BIOMARKERS AND METHODS FOR DETERMINING SENSITIVITY TO ~~EPIDERMAL~~ GROWTH FACTOR RECEPTOR MODULATORS

FIELD OF THE INVENTION

The present invention relates generally to the field of pharmacogenomics, and more specifically to methods and procedures to determine sensitivity in patients to allow the development of individualized genetic profiles which aid in treating diseases and disorders based on patient response at a molecular level.

BACKGROUND OF THE INVENTION:

Cancer is a disease with extensive histoclinical heterogeneity. Although conventional histological and clinical features have been correlated to prognosis, the same apparent prognostic type of tumors varies widely in its responsiveness to therapy and consequent survival of the patient.

New prognostic and predictive markers, which would facilitate an individualization of therapy for each patient, are needed to accurately predict patient response to treatments, such as small molecule or biological molecule drugs, in the clinic. The problem may be solved by the identification of new parameters that could better predict the patient's sensitivity to treatment. The classification of patient samples is a crucial aspect of cancer diagnosis and treatment. The association of a patient's response to a treatment with molecular and genetic markers can open up new opportunities for treatment development in non-responding patients, or distinguish a treatment's indication among other treatment choices because of higher confidence in the efficacy. Further, the pre-selection of patients who are likely to respond well to a medicine, drug, or combination therapy may reduce the number of patients needed in a clinical study or accelerate the time needed to complete a clinical development program (M. Cockett et al., 2000, *Current Opinion in Biotechnology*, 11:602-609).

The ability to predict drug sensitivity in patients is particularly challenging because drug responses reflect not only properties intrinsic to the target cells, but also a host's metabolic properties. Efforts to use genetic information to predict drug sensitivity have primarily focused on individual genes that have broad effects, such as the multidrug resistance genes, *mdr1* and *mrp1* (P. Sonneveld, 2000, *J. Intern. Med.*, 247:521-534).

The development of microarray technologies for large scale characterization of gene mRNA expression pattern has made it possible to systematically search for molecular markers and to categorize cancers into distinct subgroups not evident by traditional histopathological methods (J. Khan et al., 1998, *Cancer Res.*, 58:5009-5013; A.A. Alizadeh et al., 2000, *Nature*, 403:503-511; M. Bittner et al., 2000, *Nature*, 406:536-540; J. Khan et al., 2001, *Nature Medicine*, 7(6):673-679; and T.R. Golub et al., 1999, *Science*, 286:531-537; U. Alon et al., 1999, *Proc. Natl. Acad. Sci. USA, 96*:6745-6750). Such technologies and molecular tools have made it possible to monitor the expression level of a large number of transcripts within a cell population at any given time (see, e.g., Schena et al., 1995, *Science*, 270:467-470; Lockhart et al., 1996, *Nature Biotechnology*, 14:1675-1680; Blanchard et al., 1996, *Nature Biotechnology*, 14:1649; U.S. Patent No. 5,569,588 to Ashby et al.).

Recent studies demonstrate that gene expression information generated by microarray analysis of human tumors can predict clinical outcome (L.J. van't Veer et al., 2002, *Nature*, 415:530-536; M. West et al., 2001, *Proc. Natl. Acad. Sci. USA,* 98:11462-11467; T. Sorlie et al., 2001, *Proc. Natl. Acad. Sci. USA,* 98:10869-10874; M. Shipp et al., 2002, *Nature Medicine*, 8(1):68-74). These findings bring hope that cancer treatment will be vastly improved by better predicting the response of individual tumors to therapy.

Needed are new and alternative methods and procedures to determine drug sensitivity in patients to allow the development of individualized genetic profiles which are necessary to treat diseases and disorders based on patient response at a molecular level.

SUMMARY OF THE INVENTION:

The invention provides methods and procedures for determining patient sensitivity to one or more Epidermal Growth Factor Receptor (EGFR) modulators. The invention also provides methods of determining or predicting whether an individual requiring therapy for a disease state such as cancer will or will not respond to treatment, prior to administration of the treatment, wherein the treatment comprises one or more EGFR modulators. The one or more EGFR modulators are compounds that can be selected from, for example, one or more EGFR specific ligands, one or

more small molecule EGFR inhibitors, or one or more EGFR binding monoclonal antibodies.

In one aspect, the invention provides a method for identifying a mammal that will respond therapeutically to a method of treating cancer comprising administering an EGFR modulator, wherein the method comprises: (a) measuring in the mammal the level of at least one biomarker selected from the biomarkers of Table 4; (b) exposing the mammal to the EGFR modulator; (c) following the exposing of step (b), measuring in the mammal the level of the at least one biomarker, wherein a difference in the level of the at least one biomarker measured in step (c) compared to the level of the at least one biomarker measured in step (a) indicates that the mammal will respond therapeutically to said method of treating cancer.

As used herein, respond therapeutically refers to the alleviation or abrogation of the cancer. This means that the life expectancy of an individual affected with the cancer will be increased or that one or more of the symptoms of the cancer will be reduced or ameliorated. The term encompasses a reduction in cancerous cell growth or tumor volume. Whether a mammal responds therapeutically can be measured by many methods well known in the art, such as PET imaging.

The at least one biomarker can also be selected from the biomarkers of Table 5. The mammal can be, for example, a human, rat, mouse, dog rabbit, pig sheep, cow, horse, cat, primate, or monkey.

The method of the invention can be, for example, an in vitro method and wherein the at least one biomarker is measured in at least one mammalian biological sample from the mammal. The biological sample can comprise, for example, at least one of whole fresh blood, peripheral blood mononuclear cells, frozen whole blood, fresh plasma, frozen plasma, urine, saliva, skin, hair follicle, or tumor tissue.

In another aspect, the invention provides a method for identifying a mammal that will respond therapeutically to a method of treating cancer comprising administering an EGFR modulator, wherein the method comprises: (a) exposing the mammal to the EGFR modulator; (b) following the exposing of step (a), measuring in the mammal the level of the at least one biomarker selected from the biomarkers of Table 4, wherein a difference in the level of the at least one biomarker measured in step (b), compared to the level of the biomarker in a mammal that has not been

exposed to said EGFR modulator, indicates that the mammal will respond therapeutically to said method of treating cancer.

In yet another aspect, the invention provides a method for testing or predicting whether a mammal will respond therapeutically to a method of treating cancer comprising administering an EGFR modulator, wherein the method comprises: (a) measuring in the mammal the level of at least one biomarker selected from the biomarkers of Table 4; (b) exposing the mammal to the EGFR modulator; (c) following the exposing of step (b), measuring in the mammal the level of the at least one biomarker, wherein a difference in the level of the at least one biomarker measured in step (c) compared to the level of the at least one biomarker measured in step (a) indicates that the mammal will respond therapeutically to said method of treating cancer.

In another aspect, the invention provides a method for determining whether a compound inhibits EGFR activity in a mammal, comprising: (a) exposing the mammal to the compound; and (b) following the exposing of step (a), measuring in the mammal the level of at least one biomarker selected from the biomarkers of Table 4, wherein a difference in the level of said biomarker measured in step (b), compared to the level of the biomarker in a mammal that has not been exposed to said compound, indicates that the compound inhibits EGFR activity in the mammal.

In yet another aspect, the invention provides a method for determining whether a mammal has been exposed to a compound that inhibits EGFR activity, comprising (a) exposing the mammal to the compound; and (b) following the exposing of step (a), measuring in the mammal the level of at least one biomarker selected from the biomarkers of Table 4, wherein a difference in the level of said biomarker measured in step (b), compared to the level of the biomarker in a mammal that has not been exposed to said compound, indicates that the mammal has been exposed to a compound that inhibits EGFR activity.

In another aspect, the invention provides a method for determining whether a mammal is responding to a compound that inhibits EGFR activity, comprising (a) exposing the mammal to the compound; and (b) following the exposing of step (a), measuring in the mammal the level of at least one biomarker selected from the biomarkers of Table 4, wherein a difference in the level of said biomarker measured

in step (b), compared to the level of the biomarker in a mammal that has not been exposed to said compound, indicates that the mammal is responding to the compound that inhibits EGFR activity.

As used herein, "responding" encompasses responding by way of a biological and cellular response, as well as a clinical response (such as improved symptoms, a therapeutic effect, or an adverse event), in a mammal

The invention also provides an isolated biomarker selected from the biomarkers of Table 4. The biomarkers of the invention comprise sequences selected from the nucleotide and amino acid sequences provided in Table 4 and the Sequence Listing, as well as fragments and variants thereof.

The invention also provides a biomarker set comprising two or more biomarkers selected from the biomarkers of Table 4.

The invention also provides kits for determining or predicting whether a patient would be susceptible or resistant to a treatment that comprises one or more EGFR modulators. The patient may have a cancer or tumor such as, for example, a colon cancer or tumor.

In one aspect, the kit comprises a suitable container that comprises one or more specialized microarrays of the invention, one or more EGFR modulators for use in testing cells from patient tissue specimens or patient samples, and instructions for use. The kit may further comprise reagents or materials for monitoring the expression of a biomarker set at the level of mRNA or protein.

In another aspect, the invention provides a kit comprising two or more biomarkers selected from the biomarkers of Table 4.

In yet another aspect, the invention provides a kit comprising at least one of an antibody and a nucleic acid for detecting the presence of at least one of the biomarkers selected from the biomarkers of Table 4. In one aspect, the kit further comprises instructions for determining whether or not a mammal will respond therapeutically to a method of treating cancer comprising administering a compound that inhibits EGFR activity. In another aspect, the instructions comprise the steps of (a) measuring in the mammal the level of at least one biomarker selected from the biomarkers of Table 4, (b) exposing the mammal to the compound, (c) following the exposing of step (b), measuring in the mammal the level of the at least one biomarker,

wherein a difference in the level of the at least one biomarker measured in step (c) compared to the level of the at least one biomarker measured in step (a) indicates that the mammal will respond therapeutically to said method of treating cancer.

The invention also provides screening assays for determining if a patient will be susceptible or resistant to treatment with one or more EGFR modulators.

The invention also provides a method of monitoring the treatment of a patient having a disease treatable by one or more EGFR modulators.

The invention also provides individualized genetic profiles which are necessary to treat diseases and disorders based on patient response at a molecular level.

The invention also provides specialized microarrays, e.g., oligonucleotide microarrays or cDNA microarrays, comprising one or more biomarkers having expression profiles that correlate with either sensitivity or resistance to one or more EGFR modulators.

The invention also provides antibodies, including polyclonal or monoclonal, directed against one or more biomarkers of the invention.

The invention will be better understood upon a reading of the detailed description of the invention when considered in connection with the accompanying figures.

BRIEF DESCRIPTION OF THE FIGURES:

FIG. 1 illustrates a EGFR biomarker identification and prioritization strategy.

FIG. 2A illustrates the RT-PCR results for EGFR in thirty one colon cancer cell lines to identify cell lines which do not have significant mRNA expression of EGFR.

FIG. 2B illustrates the IC_{50} profile for twenty two colon cancer cell lines with an EGFR inhibitor compound, and determination of sensitive and resistant cell lines.

DETAILED DESCRIPTION OF THE INVENTION:

The invention provides biomarkers that respond to the modulation of a specific signal transduction pathway and also correlate with EGFR modulator sensitivity or resistance. These biomarkers can be employed for predicting response to one or more EGFR modulators. In one aspect, the biomarkers of the invention are those provided in Table 4 and the Sequence Listing, including both polynucleotide and polypeptide sequences.

The biomarkers were determined by an *in vitro* assay employing microarray technology to monitor simultaneously the expression pattern of thousands of discrete genes in untreated cells, whose response to the modulation of a signal transduction pathway, in particular the EGFR pathway, was tested on untreated cells whose sensitivity to EGFR modulators was tested. The biomarkers have expression levels in the cells that are dependent on the activity of the EFGR signal transduction pathway and that are also highly correlated with EGFR modulator sensitivity exhibited by the cells. Biomarkers serve as useful molecular tools for predicting a response to EGFR modulators, preferably biological molecules, small molecules, and the like that affect EGFR kinase activity via direct or indirect inhibition or antagonism of EGFR kinase function or activity.

EGFR MODULATORS

As used herein, the term "EGFR modulator" is intended to mean a compound or drug that is a biological molecule or a small molecule that directly or indirectly modulates EGFR activity or the EGFR signal transduction pathway. Thus, compounds or drugs as used herein is intended to include both small molecules and biological molecules. Direct or indirect modulation includes activation or inhibition of EGFR activity or the EGFR signal transduction pathway. In one aspect, inhibition refers to inhibition of the binding of EGFR to an EGFR ligand such as, for example, EGF. In another aspect, inhibition refers to inhibition of the kinase activity of EGFR.

EGFR modulators include, for example, EGFR specific ligands, small molecule EGFR inhibitors, and EGFR monoclonal antibodies. In one aspect, the EGFR modulator inhibits EGFR activity and/or inhibits the EGFR signal transduction

pathway. In another aspect, the EGFR modulator is an EGFR antibody that inhibits EGFR activity and/or inhibits the EGFR signal transduction pathway.

EGFR modulators include biological molecules or small molecules. Biological molecules include all lipids and polymers of monosaccharides, amino acids, and nucleotides having a molecular weight greater than 450. Thus, biological molecules include, for example, oligosaccharides and polysaccharides; oligopeptides, polypeptides, peptides, and proteins; and oligonucleotides and polynucleotides. Oligonucleotides and polynucleotides include, for example, DNA and RNA.

Biological molecules further include derivatives of any of the molecules described above. For example, derivatives of biological molecules include lipid and glycosylation derivatives of oligopeptides, polypeptides, peptides, and proteins.

Derivatives of biological molecules further include lipid derivatives of oligosaccharides and polysaccharides, e.g., lipopolysaccharides. Most typically, biological molecules are antibodies, or functional equivalents of antibodies. Functional equivalents of antibodies have binding characteristics comparable to those of antibodies, and inhibit the growth of cells that express EGFR. Such functional equivalents include, for example, chimerized, humanized, and single chain antibodies as well as fragments thereof.

Functional equivalents of antibodies also include polypeptides with amino acid sequences substantially the same as the amino acid sequence of the variable or hypervariable regions of the antibodies. An amino acid sequence that is substantially the same as another sequence, but that differs from the other sequence by means of one or more substitutions, additions, and/or deletions, is considered to be an equivalent sequence. Preferably, less than 50%, more preferably less than 25%, and still more preferably less than 10%, of the number of amino acid residues in a sequence are substituted for, added to, or deleted from the protein.

The functional equivalent of an antibody is preferably a chimerized or humanized antibody. A chimerized antibody comprises the variable region of a non-human antibody and the constant region of a human antibody. A humanized antibody comprises the hypervariable region (CDRs) of a non-human antibody. The variable region other than the hypervariable region, e.g., the framework variable region, and the constant region of a humanized antibody are those of a human antibody.

Suitable variable and hypervariable regions of non-human antibodies may be derived from antibodies produced by any non-human mammal in which monoclonal antibodies are made. Suitable examples of mammals other than humans include, for example, rabbits, rats, mice, horses, goats, or primates.

Functional equivalents further include fragments of antibodies that have binding characteristics that are the same as, or are comparable to, those of the whole antibody. Suitable fragments of the antibody include any fragment that comprises a sufficient portion of the hypervariable (i.e., complementarity determining) region to bind specifically, and with sufficient affinity, to EGFR tyrosine kinase to inhibit growth of cells that express such receptors.

Such fragments may, for example, contain one or both Fab fragments or the $F(ab')_2$ fragment. Preferably, the antibody fragments contain all six complementarity determining regions of the whole antibody, although functional fragments containing fewer than all of such regions, such as three, four, or five CDRs, are also included.

In one aspect, the fragments are single chain antibodies, or Fv fragments. Single chain antibodies are polypeptides that comprise at least the variable region of the heavy chain of the antibody linked to the variable region of the light chain, with or without an interconnecting linker. Thus, Fv fragment comprises the entire antibody combining site. These chains may be produced in bacteria or in eukaryotic cells.

The antibodies and functional equivalents may be members of any class of immunoglobulins, such as IgG, IgM, IgA, IgD, or IgE, and the subclasses thereof. In one aspect, the antibodies are members of the IgG1 subclass. The functional equivalents may also be equivalents of combinations of any of the above classes and subclasses.

In one aspect, EGFR antibodies can be selected from chimerized, humanized, fully human, and single chain antibodies derived from the murine antibody 225 described in U.S. Patent No. 4,943,533 to Mendelsohn et al. In one aspect, the 225 derived antibodies have the following hypervariable (CDR) regions of the light and heavy chain, wherein the amino acid sequences are indicated below the nucleotide sequences:

HEAVY CHAIN HYPERVARIABLE REGIONS (VH):

CDR1

AACTATGGTGTACAC (SEQ ID NO: 179)

N Y G V H (SEQ ID NO: 180)

CDR2

GTGATATGGAGTGGTGGAAACACAGACTATAATACACCTTTCACATCC (SEQ ID NO: 181)

V I W S G G N T D Y N T P F T S (SEQ ID NO: 182)

CDR3

GCCCTCACCTACTATGATTACGAGTTTGCTTAC (SEQ ID NO: 183)

A L T Y Y D Y E F A Y (SEQ ID NO: 184)

LIGHT CHAIN HYPERVARIABLE REGIONS (VL):

CDR1

AGGGCCAGTCAGAGTATTGGCACAAACATACAC (SEQ ID NO: 185)

R A S Q S I G T N I H (SEQ ID NO: 186)

CDR2

GCTTCTGAGTCTATCTCT (SEQ ID NO: 187)

A S E S I S (SEQ ID NO: 188)

CDR3

CAACAAAATAATAACTGGCCAACCACG (SEQ ID NO: 189)

Q Q N N N W P T T (SEQ ID NO: 190)

In another aspect, the EGFR antibody can be selected from the antibodies described in U.S. Patent No. 6,235,883 to Jakobovits et al., U.S. Patent No. 5,558,864 to Bendi et al., and U.S. Patent No. 5,891,996 to Mateo de Acosta del Rio et al.

In addition to the biological molecules discussed above, the EGFR modulators useful in the invention may also be small molecules. Any molecule that is not a biological molecule is considered herein to be a small molecule. Some examples of small molecules include organic compounds, organometallic compounds, salts of organic and organometallic compounds, saccharides, amino acids, and nucleotides. Small molecules further include molecules that would otherwise be considered biological molecules, except their molecular weight is not greater than 450. Thus,

small molecules may be lipids, oligosaccharides, oligopeptides, and oligonucleotides and their derivatives, having a molecular weight of 450 or less.

It is emphasized that small molecules can have any molecular weight. They are merely called small molecules because they typically have molecular weights less than 450. Small molecules include compounds that are found in nature as well as synthetic compounds. In one embodiment, the EGFR modulator is a small molecule that inhibits the growth of tumor cells that express EGFR. In another embodiment, the EGFR modulator is a small molecule that inhibits the growth of refractory tumor cells that express EGFR.

Numerous small molecules have been described as being useful to inhibit EGFR. For example, U.S. Patent No. 5,656,655 to Spada et al. discloses styryl substituted heteroaryl compounds that inhibit EGFR. The heteroaryl group is a monocyclic ring with one or two heteroatoms, or a bicyclic ring with 1 to about 4 heteroatoms, the compound being optionally substituted or polysubstituted.

U.S. Patent No. 5,646,153 to Spada et al. discloses bis mono and/or bicyclic aryl heteroaryl, carbocyclic, and heterocarbocyclic compounds that inhibit EGFR.

U.S. Patent No. 5,679,683 to Bridges et al. discloses tricyclic pyrimidine compounds that inhibit the EGFR. The compounds are fused heterocyclic pyrimidine derivatives described at column 3, line 35 to column 5, line 6.

U.S. Patent No. 5,616,582 to Barker discloses quinazoline derivatives that have receptor tyrosine kinase inhibitory activity.

Fry et al., Science 265, 1093-1095 (1994) in Figure 1 discloses a compound having a structure that inhibits EGFR.

Osherov et al. disclose tyrphostins that inhibit EGFR/HER1 and HER 2, particularly those in Tables I, II, III, and IV.

U.S. Patent No. 5,196,446 to Levitzki et al. discloses heteroarylethenediyl or heteroarylethendeiylaryl compounds that inhibit EGFR, particularly from column 2, line 42 to column 3, line 40.

Panek et al., Journal of Pharmacology and Experimental Therapeutics 283, 1433-1444 (1997) discloses a compound identified as PD166285 that inhibits the EGFR, PDGFR, and FGFR families of receptors. PD166285 is identified as 6-(2,6-

dichlorophenyl)-2-(4-(2-diethylaminoethyoxy)phenylamino)-8-methyl-8H-pyrido(2,3-d)pyrimidin-7-one having the structure shown in Figure 1 on page 1436.

BIOMARKERS AND BIOMARKER SETS

The invention includes individual biomarkers and biomarker sets having both diagnostic and prognostic value in disease areas in which signaling through EGFR or the EGFR pathway is of importance, e.g., in cancers or tumors, in immunological disorders, conditions or dysfunction, or in disease states in which cell signaling and/or cellular proliferation controls are abnormal or aberrant. The biomarker sets comprise a plurality of biomarkers such as, for example, a plurality of the biomarkers provided in Table 4 below, that highly correlate with resistance or sensitivity to one or more EGFR modulators.

The biomarker sets of the invention enable one to predict or reasonably foretell the likely effect of one or more EGFR modulators in different biological systems or for cellular responses. The biomarker sets can be used in *in vitro* assays of EGFR modulator response by test cells to predict *in vivo* outcome. In accordance with the invention, the various biomarker sets described herein, or the combination of these biomarker sets with other biomarkers or markers, can be used, for example, to predict how patients with cancer might respond to therapeutic intervention with one or more EGFR modulators.

A biomarker set of cellular gene expression patterns correlating with sensitivity or resistance of cells following exposure of the cells to one or more EGFR modulators provides a useful tool for screening one or tumor samples before treatment with the EGFR modulator. The screening allows a prediction of cells of a tumor sample exposed to one or more EGFR modulators, based on the expression results of the biomarker set, as to whether or not the tumor, and hence a patient harboring the tumor, will or will not respond to treatment with the EGFR modulator.

The biomarker or biomarker set can also be used as described herein for monitoring the progress of disease treatment or therapy in those patients undergoing treatment for a disease involving an EGFR modulator.

The biomarkers serve as targets for the development of therapies for disease treatment. Such targets may be particularly applicable to treatment of breast disease,

such as breast cancers or tumors. Indeed, because these biomarkers are differentially expressed in sensitive and resistant cells, their expression patterns are correlated with relative intrinsic sensitivity of cells to treatment with EGFR modulators. Accordingly, the biomarkers highly expressed in resistant cells may serve as targets for the development of new therapies for the tumors which are resistant to EGFR modulators, particularly EGFR inhibitors.

MICROARRAYS

The invention also includes specialized microarrays, e.g., oligonucleotide microarrays or cDNA microarrays, comprising one or more biomarkers, showing expression profiles that correlate with either sensitivity or resistance to one or more EGFR modulators. Such microarrays can be employed in *in vitro* assays for assessing the expression level of the biomarkers in the test cells from tumor biopsies, and determining whether these test cells are likely to be resistant or sensitive to EGFR modulators. For example, a specialized microarray can be prepared using all the biomarkers, or subsets thereof, as described herein and shown in Table 4. Cells from a tissue or organ biopsy can be isolated and exposed to one or more of the EGFR modulators. Following application of nucleic acids isolated from both untreated and treated cells to one or more of the specialized microarrays, the pattern of gene expression of the tested cells can be determined and compared with that of the biomarker pattern from the control panel of cells used to create the biomarker set on the microarray. Based upon the gene expression pattern results from the cells that underwent testing, it can be determined if the cells show a resistant or a sensitive profile of gene expression. Whether or not the tested cells from a tissue or organ biopsy will respond to one or more of the EGFR modulators and the course of treatment or therapy can then be determined or evaluated based on the information gleaned from the results of the specialized microarray analysis.

ANTIBODIES

The invention also includes antibodies, including polyclonal or monoclonal, directed against one or more of the polypeptide biomarkers. Such antibodies can be used in a variety of ways, for example, to purify, detect, and target the biomarkers of

the invention, including both *in vitro* and *in vivo* diagnostic, detection, screening, and/or therapeutic methods.

KITS

The invention also includes kits for determining or predicting whether a patient would be susceptible or resistant to a treatment that comprises one or more EGFR modulators. The patient may have a cancer or tumor such as, for example, a breast cancer or tumor. Such kits would be useful in a clinical setting for use in testing a patient's biopsied tumor or cancer samples, for example, to determine or predict if the patient's tumor or cancer will be resistant or sensitive to a given treatment or therapy with an EGFR modulator. The kit comprises a suitable container that comprises: one or more microarrays, e.g., oligonucleotide microarrays or cDNA microarrays, that comprise those biomarkers that correlate with resistance and sensitivity to EGFR modulators, particularly EGFR inhibitors; one or more EGFR modulators for use in testing cells from patient tissue specimens or patient samples; and instructions for use. In addition, kits contemplated by the invention can further include, for example, reagents or materials for monitoring the expression of biomarkers of the invention at the level of mRNA or protein, using other techniques and systems practiced in the art such as, for example, RT-PCR assays, which employ primers designed on the basis of one or more of the biomarkers described herein, immunoassays, such as enzyme linked immunosorbent assays (ELISAs), immunoblotting, e.g., Western blots, or *in situ* hybridization, and the like, as further described herein.

APPLICATION OF BIOMARKERS AND BIOMARKER SETS

The biomarkers and biomarker sets may be used in different applications. Biomarker sets can be built from any combination of biomarkers listed in Table 4 to make predictions about the likely effect of any EGFR modulator in different biological systems. The various biomarkers and biomarker sets described herein can be used, for example, as diagnostic or prognostic indicators in disease management, to predict how patients with cancer might respond to therapeutic intervention with compounds that modulate the EGFR, and to predict how patients might respond to

therapeutic intervention that modulates signaling through the entire EGFR regulatory pathway.

While the data described herein were generated in cell lines that are routinely used to screen and identify compounds that have potential utility for cancer therapy, the biomarkers have both diagnostic and prognostic value in other diseases areas in which signaling through EGFR or the EGFR pathway is of importance, e.g., in immunology, or in cancers or tumors in which cell signaling and/or proliferation controls have gone awry.

In the examples described below, the sensitivity and resistance classifications in the twenty two colon cell lines were similar for the two EGFR modulators tested. Therefore, the biomarkers of the invention are expected to have both diagnostic and prognostic value for other compounds that modulate EGFR or the EGFR signaling pathways.

Those having skill in the pertinent art will appreciate that the EGFR signaling pathway is used and functional in cell types other than cell lines of colon tissue. Therefore, the described biomarkers are expected to have utility for predicting drug sensitivity or resistance to compounds that interact with or inhibit the EGFR activity in cells from other tissues or organs associated with a disease state, or cancers or tumors derived from other tissue types. Non-limiting examples of such cells, tissues and organs include breast, colon, lung, prostate, testes, ovaries, cervix, esophagus, pancreas, spleen, liver, kidney, stomach, lymphocytic and brain, thereby providing a broad and advantageous applicability to the biomarkers described herein. Cells for analysis can be obtained by conventional procedures as known in the art, for example, tissue biopsy, aspiration, sloughed cells, e.g., colonocytes, clinical or medical tissue or cell sampling procedures.

In accordance with the invention, cells from a patient tissue sample, e.g., a tumor or cancer biopsy, can be assayed to determine the expression pattern of one or more biomarkers prior to treatment with one or more EGFR modulators. Success or failure of a treatment can be determined based on the biomarker expression pattern of the cells from the test tissue (test cells), e.g., tumor or cancer biopsy, as being relatively similar or different from the expression pattern of a control set of the one or more biomarkers. Thus, if the test cells show a biomarker expression profile which

corresponds to that of the biomarkers in the control panel of cells which are sensitive to the EGFR modulator, it is highly likely or predicted that the individual's cancer or tumor will respond favorably to treatment with the EGFR modulator. By contrast, if the test cells show a biomarker expression pattern corresponding to that of the biomarkers of the control panel of cells which are resistant to the EGFR modulator, it is highly likely or predicted that the individual's cancer or tumor will not respond to treatment with the EGFR modulator.

The invention also provides a method of monitoring the treatment of a patient having a disease treatable by one or more EGFR modulators. The isolated test cells from the patient's tissue sample, e.g., a tumor biopsy or tumor sample, can be assayed to determine the expression pattern of one or more biomarkers before and after exposure to an EGFR modulator wherein, preferably, the EGFR modulator is an EGFR inhibitor. The resulting biomarker expression profile of the test cells before and after treatment is compared with that of one or more biomarkers as described and shown herein to be highly expressed in the control panel of cells that are either resistant or sensitive to an EGFR modulator. Thus, if a patient's response is sensitive to treatment by an EGFR modulator, based on correlation of the expression profile of the one or biomarkers, the patient's treatment prognosis can be qualified as favorable and treatment can continue. Also, if, after treatment with an EGFR modulator, the test cells don't show a change in the biomarker expression profile corresponding to the control panel of cells that are sensitive to the EGFR modulator, it can serve as an indicator that the current treatment should be modified, changed, or even discontinued. This monitoring process can indicate success or failure of a patient's treatment with an EGFR modulator and such monitoring processes can be repeated as necessary or desired.

The biomarkers of the invention can be used to predict an outcome prior to having any knowledge about a biological system. Essentially, a biomarker can be considered to be a statistical tool. Biomarkers are useful primarily in predicting the phenotype that is used to classify the biological system. In an embodiment of the invention, the goal of the prediction is to classify cancer cells as having an active or inactive EGFR pathway. Cancer cells with an inactive EGFR pathway can be considered resistant to treatment with an EGFR modulator. An inactive EGFR

pathway is defined herein as a non-significant expression of the EGFR or by a classification as "resistant" or "sensitive" based on the IC_{50} value of each colon cell line to a compound (EGFR inhibitor compound BMS-461453) exemplified herein.

A number of the biomarker described herein are known to be regulated by EGFR, e.g., mucin 2 (J Biol Chem. 2002 Aug 30;277(35):32258-67). Another biomarker, betacellulin, is know to be an EGFR ligand (Biochem Biophys Res Commun. 2002 Jun 28;294(5):1040-6). A functional relationship of the top biomarkers to the EGFR is expected, since biomarkers that contribute to high biomarker accuracy are likely to play a functional role in the pathway that is being modulated. For example, Perception therapy (i.e., antibody that binds to the Her2 receptor and prevents function via internalization) is indicated when the Her2 gene is overexpressed. It is unlikely that a therapy will have any therapeutic effect if the target enzyme is not expressed.

However, although the complete function of all of the biomarkers are not currently known, some of the biomarkers are likely to be directly or indirectly involved in the EGFR signaling pathway. In addition, some of the biomarkers may function in the metabolic or other resistance pathways specific to the EGFR modulators tested. Notwithstanding, knowledge about the function of the biomarkers is not a requisite for determining the accuracy of a biomarker according to the practice of the invention.

DISCOVERY OF BIOMARKERS

An approach has been discovered in which biomarkers were identified whose expression patterns, in a subset of cell lines, correlated to and can be used as an *in vitro* marker of cellular response to treatment or therapy with one compound, or with a combination or series of compounds, that are known to inhibit or activate the function of a protein, enzyme, or molecule (e.g., a receptor) that is directly or indirectly involved in cell proliferation, cell responses to external stimuli, (such as ligand binding), or signal transduction, e.g., a receptor tyrosine kinase. Preferred are antagonists or inhibitors of the function of a given protein, e.g., a receptor tyrosine kinase.

Two analytical strategies were deployed to discover biomarkers useful for predicting the sensitivity or resistance of cancer cells to treatment with one or more EGFR modulators. FIG. 1 illustrates the EGFR biomarker identification and prioritization strategy. In one strategy, the mRNA expression level of EGFR was used to identify six colon cancer cell lines with, inferred from the mRNA expression level, no significant presence of the EGFR protein and hence no significant activity of the EGFR pathway (FIG. 2A). In subsequent analyses, biomarkers were identified that had no significant mRNA expression level in the six cell lines and no inferred presence of the EGFR protein. Further, it was required that these biomarkers would have a significant mRNA expression level in at least six other cell lines.

In a second strategy, an EGFR specific tyrosine kinase inhibitor compound was used to determine compound sensitivity in a panel of twenty two colon cancer cell lines following exposure of the cells to the compound. Some of the cell lines were determined to be resistant to treatment with the inhibitor compound, while others were determined to be sensitive to the inhibitor (FIG. 2B). A subset of the cell lines examined provided an expression pattern or profile of biomarkers that correlated to a response by the cells to the EGFR inhibitor compound as well as the absence of significant EGFR expression as thus could serve as biomarkers.

By combining the use of EGFR co-regulation studies in tumor cells with experimental studies in cultured cells as a model of *in vivo* effects, the invention advantageously focuses on cell-intrinsic properties that are exposed in cell culture to identify biomarkers that predict compound sensitivity and resistance. The discovery and identification of biomarkers in tumor cells and cell lines assayed *in vitro* can be used to predict responses to one or more EGFR modulators *in vivo* and, thus, can be extended to clinical situations in which the same biomarkers are used to predict patients' responses to one or more EGFR modulators and treatments comprising one or more EGFR modulators.

As described in the examples below, oligonucleotide microarrays were used to measure the expression levels of over 44,792 probe sets in a panel of thirty one untreated colon cancer cell lines for which the expression status of the EGFR and the drug sensitivity to EGFR inhibitor compounds was determined. This analysis was performed to determine whether the gene expression signatures of untreated cells

were sufficient for the prediction of sensitivity of the disease to inhibition of the EGFR by small molecule or biological molecule compounds. Through data analysis, biomarkers were identified whose expression levels were found to be highly counter-correlated with the status of the EGFR and correlated with the drug sensitivity. In addition, the treatment of cells with a small molecule EGFR inhibitor also provided gene expression signatures predictive of sensitivity to the compound.

The means of performing the gene expression and biomarker identification analyses embraced by the invention is described in further detail and without limitation below.

IC_{50} Determination and Phenotype Classification Based on Sensitivity of Twenty-two Colon Cancer Cell lines to EGFR Inhibitor Compounds

Twenty two colon cell lines were treated with a small molecule EGFR inhibitor (BMS-461453) to determine the individual IC_{50} value. The IC_{50} for each cell line was assessed by MTS assays. The average IC_{50} values along with standard deviations were calculated from two to five individual determinations for each cell line. As shown in FIG. 2B, a 4-fold variation in the IC_{50} values was observed for the small molecule EGFR inhibitor among the 22 colon cancer cell lines. The IC_{50} unit is μM.

All cell lines with at least a 1.75 fold lower IC_{50} than the most resistant cell lines were considered to be sensitive to treatment with the small molecule EGFR inhibitor. FIG. 2B represents the resistance/sensitivity classifications of the twenty-two colon cell lines to the small molecule EGFR inhibitor. Five cell lines were classified as sensitive and seventeen cell lines as resistant.

Description of the Strategy for Identifying Biomarkers

Biomarkers were discovered based on two criteria: (i) the correlation of their mRNA expression level to the expression of EGFR in cell lines with insignificant EGFR expression and (ii) the correlation of the IC_{50} values for the small molecule EGFR inhibitor BMS-461453 with gene expression levels.

For each of these two biomarker selection strategies, two independent "discovery" probe set lists were established by using statistical filters with different

stringency levels to identify genes whose expression correlated with either EGFR status or IC_{50} value. These statistical methods are described below and resulted in four discovery probe set lists: EGFR-A and EGFR-B (correlation with no significant EGFR expression) and IC-50-A, IC-50-B (correlation with IC_{50} expression), the A-lists containing probe sets selected by more stringent conditions. To then establish two biomarker probe set lists, probe sets that appeared in both EGFR-A and IC-50 B were selected (Biomarker Probe Set List A, Table 2) and probe sets that appeared in both EGFR-B and IC-50-A were selected (Biomarker Probe Set List B, Table 3).

Identifying Genes that Significantly Correlate with EGFR status classification

RT-PCR expression data for EGFR were obtained from thirty one colon cancer cell lines and six cell lines with a significantly lower expression level of EGFR compared to the other cell lines were identified as described in Example 1 below. (FIG. 2A). Expression profiling data of 44,792 probe sets represented on the HG-U133 array set for all thirty one untreated colon cancer cell lines were obtained and analyzed for the identification of probe sets which would be correlated with the above described six cell lines with no significant mRNA expression of EGFR. For the discovery probe set list EGFR-A, all probe sets which were judged to be absent by the Affymetrix Mas 5.0 software in six of the six colon cancer cell lines with significantly lower expression of EGFR were identified. Second, it was required that these probe sets would be judged to be present in at least six cell lines of the twenty five cell lines classified as having significant mRNA expression of the EGFR. This analytical strategy resulted in the identification of 280 probe sets that could be analyzed in comparison to the discovery probe set list IC-50-B.

The discovery probe set list EGFR-B was generated by selecting all probe sets which were judged to be absent by the Affymetrix Mas 5.0 software in five of the six colon cancer cell lines with significantly lower expression of EGFR and which would be present in at least six cell lines of the twenty five cell lines classified as having significant mRNA expression of the EGFR. Discovery probe set list EGR-B contains 1,852 probe sets (U133A: 876; U133B: 976).

Identifying Genes that Significantly Correlate with Drug Resistance/Sensitivity Classification

Expression profiling data of 44,792 probe sets represented on the HG-U133 array set for twenty two untreated colon cell lines were obtained and preprocessed as described in Example 1 below. These data were analyzed using the Student's TTEST to identify genes whose expression patterns were strongly correlated with the drug resistance/sensitivity classification. Table 1 provides the resistance/sensitivity phenotype classification of the twenty two colon cell lines for the EGFR antagonist BMS-461453 based on the IC_{50} results. The mean IC_{50} values along with standard deviations (SD) were calculated from 2 to 5 individual determinations for each cell line as shown. The mean IC_{50} across the twenty two colon cell lines for BMS-461453 was calculated and used to normalize the IC_{50} data for each cell line. All cell lines with at least a 1.75 fold lower IC_{50} than the most resistant cell lines were considered to be sensitive to treatment with BMS-461453. The cell lines designated with an asterisk are defined as being sensitive to the drug treatment.

TABLE 1 - Resistance/Sensitivity Phenotype Classification of Twenty Two Colon Cell Lines

Cell lines	IC_{50} (μM)	SD
CCD_33C0*	2	1.28
LOVO*	2.3	2.28
*LS174T**	3.5	1.93
Caco2*	5.5	3.97
SW403*	5.7	4.94
CCD18Co	7.1	3.84
SW837	7.2	3.30
Sk-Co-1	9	2.02
MIP	9.7	0.52
SW1417	10	0.00
HT-29	10	0.00
T84	10	0.00
CX-1	10	0.00
Colo-205	10	0.00
Colo-201	10	0.00
Colo320HSR	10	0.00
HCT8	10	0.00
Colo320DM	10	0.00
SW480	10	0.00
HCT116	10	0.00
SW620	10	0.00
HCT116S542	10	0.00

An "idealized expression pattern" corresponds to a gene that is uniformly high in one class (e.g., sensitive) and uniformly low in the other (e.g., resistant). Initially, a Student TTEST was performed in which a T value was obtained for each probe set. Once a T value was generated, its corresponding confidence value (P) was found on a standard table of significance. The confidence value is a measure of the probability to observe a certain mean expression difference between two groups by chance alone and is obtained using the following formula:

$$T(g.c) = (X_1 - X_2) / (var_1/n_1 + var_2/n_2)^{1/2}$$

wherein,

T(g,c) represents the T value between expression for gene g and the sensitivity/resistance classification c;

X_1 represents mean gene expression level of samples in class 1;

X_2 represents mean gene expression level of samples in class 2;

var_1 represents variance of gene expression for samples in class 1;

var_2 represents variance of gene expression for samples in class 2;

n_1 represents number of samples in class 1;

n_2 represents number of samples in class 2; and

corresponding confidence value (P) for T values are obtained from a standard table of significance.

To generate discovery probe set list IC-50-B, a confidence value of 0.05 or lower was used as the cut off for probe sets to be included in the list. Discovery probe set list IC-50-B contains 5,050 probe sets (U133A: 2,498; U133B: 2,552).

Discovery probe set list IC-50-A was generated using the Pearson correlation coefficient (a dimensionless index that ranges from -1.0 to 1.0). This value was calculated by treating the IC_{50} data as continuous variables and by utilizing a linear regression model to correlate gene expression levels with IC_{50} values for twenty-two colon cell lines. Probe sets with a correlation coefficient less than –0.5 were selected ($p < 0.02$), a total of 902 probe sets (U133A: 467; U133B: 435).

Finally, two separate biomarker probe set lists were generated, biomarker probe set lists A and B, by identifying probe sets which were present in EGFR-A and IC-50-B (Biomarker Probe Set List A) (Table 2) or were present in EGFR-B and IC-50-A (Biomarker Probe Set List B) (Table 3).

The biomarker probe set list A (Table 2) contains a total of 74 probe sets (U133A: 43; U133B: 31) and provides the polynucleotides identified to be biomarkers of EGFR antagonist sensitivity employing strategy A. With strategy A, polynucleotides were required to satisfy a stringent criteria for EGFR status coregulation and a less stringent condition for correlation to IC_{50} values. Namely, the polynucleotides had to be called absent by the Affymetrix software in six out of the

six cell lines with lowest expression of EGFR and be differentially expressed in the sensitive and resistance cell lines with a P value equal to or less than 0.05.

TABLE 2 - Biomarker Probe Set List A

Unigene Title	Affymetrix Description	Affymetrix probe set
hemoglobin, alpha 1	gb:BC005931.1 /DEF=Homo sapiens, hemoglobin, alpha 2, clone MGC:14541, mRNA, complete cds. /FEA=mRNA /PROD=hemoglobin, alpha 2 /DB_XREF=gi:13543547 /FL=gb:BC005931.1	211745_x_at
dipeptidylpeptidase IV (CD26, adenosine deaminase complexing protein 2)	gb:M80536.1 /DEF=H.sapiens dipeptidyl peptidase IV (DPP4) mRNA, complete cds. /FEA=mRNA /GEN=DPP4 /PROD=dipeptidyl peptidase IV /DB_XREF=gi:181569 /UG=Hs.44926 dipeptidylpeptidase IV (CD26, adenosine deaminase complexing protein 2) /FL=gb:M80536.1 gb:NM_001935.1	203716_s_at
spondin 1, (f-spondin) extracellular matrix protein	Consensus includes gb:AI885290 /FEA=EST /DB_XREF=gi:5590454 /DB_XREF=est:wl92a04.x1 /CLONE=IMAGE:2432334 /UG=Hs.5378 spondin 1, (f-spondin) extracellular matrix protein	213994_s_at
3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2 (mitochondrial)	gb:NM_005518.1 /DEF=Homo sapiens 3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2 (mitochondrial) (HMGCS2), mRNA. /FEA=mRNA /GEN=HMGCS2 /PROD=3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2(mitochondrial) /DB_XREF=gi:5031750 /UG=Hs.59889 3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2 (mitochondrial) /FL=gb:NM_005518.1	204607_at
mucin 2, intestinal/trachea l	gb:NM_002457.1 /DEF=Homo sapiens mucin 2, intestinaltracheal (MUC2), mRNA. /FEA=mRNA /GEN=MUC2 /PROD=mucin 2, intestinaltracheal /DB_XREF=gi:4505284 /UG=Hs.315 mucin 2, intestinaltracheal /FL=gb:NM_002457.1 gb:L21998.1	204673_at
cystic fibrosis transmembrane conductance regulator, ATP-binding cassette (sub-family C, member 7)	gb:NM_000492.2 /DEF=Homo sapiens cystic fibrosis transmembrane conductance regulator, ATP-binding cassette (sub-family C, member 7) (CFTR), mRNA. /FEA=mRNA /GEN=CFTR /PROD=cystic fibrosis transmembrane conductanceregulator, ATP-binding cassette (sub-family C, member 7) /DB_XREF=gi:6995995	205043_at

	/UG=Hs.663 cystic fibrosis transmembrane conductance regulator, ATP-binding cassette (sub-family C, member 7) /FL=gb:NM_000492.2	
CUG triplet repeat, RNA-binding protein 2	Consensus includes gb:N36839 /FEA=EST /DB_XREF=gi:1157981 /DB_XREF=est:yy35f07.s1 /CLONE=IMAGE:273253 /UG=Hs.211610 CUG triplet repeat, RNA-binding protein 2 /FL=gb:U69546.1 gb:AF036956.1 gb:AF090694.1 gb:NM_006561.1	202156_s_at
nuclear receptor subfamily 3, group C, member 2	gb:NM_000901.1 /DEF=Homo sapiens nuclear receptor subfamily 3, group C, member 2 (NR3C2), mRNA. /FEA=mRNA /GEN=NR3C2 /PROD=nuclear receptor subfamily 3, group C, member 2 /DB_XREF=gi:4505198 /UG=Hs.1790 nuclear receptor subfamily 3, group C, member 2 /FL=gb:M16801.1 gb:NM_000901.1	205259_at
cystic fibrosis transmembrane conductance regulator, ATP-binding cassette (sub-family C, member 7)	Consensus includes gb:W60595 /FEA=EST /DB_XREF=gi:1367354 /DB_XREF=est:zc91b04.s1 /CLONE=IMAGE:338479 /UG=Hs.663 cystic fibrosis transmembrane conductance regulator, ATP-binding cassette (sub-family C, member 7)	215702_s_at
cytochrome P450, subfamily IIJ (arachidonic acid epoxygenase) polypeptide 2	gb:NM_000775.1 /DEF=Homo sapiens cytochrome P450, subfamily IIJ (arachidonic acid epoxygenase) polypeptide 2 (CYP2J2), mRNA. /FEA=mRNA /GEN=CYP2J2 /PROD=cytochrome P450, subfamily IIJ (arachidonic acidepoxygenase) polypeptide 2 /DB_XREF=gi:4503226 /UG=Hs.152096 cytochrome P450, subfamily IIJ (arachidonic acid epoxygenase) polypeptide 2 /FL=gb:U37143.1 gb:NM_000775.1	205073_at
cystatin S	gb:NM_001899.1 /DEF=Homo sapiens cystatin S (CST4), mRNA. /FEA=mRNA /GEN=CST4 /PROD=cystatin S /DB_XREF=gi:4503108 /UG=Hs.56319 cystatin S /FL=gb:NM_001899.1	206994_at
spondin 1, (f-spondin) extracellular matrix protein	Consensus includes gb:AI885290 /FEA=EST /DB_XREF=gi:5590454 /DB_XREF=est:wl92a04.x1 /CLONE=IMAGE:2432334 /UG=Hs.5378 spondin 1, (f-spondin) extracellular matrix protein	213993_at
fibroblast growth factor receptor 2 (bacteria-expressed kinase,	gb:NM_022969.1 /DEF=Homo sapiens fibroblast growth factor receptor 2 (bacteria-expressed kinase, keratinocyte growth factor receptor, craniofacial dysostosis 1, Crouzon syndrome,	203638_s_at

keratinocyte growth factor receptor, craniofacial dysostosis 1, Crouzon syndrome, Pfeiffer syndrome, Jackson-Weiss syndrome)	Pfeiffer syndrome, Jackson-Weiss syndrome) (FGFR2), transcript variant 2, mRNA. /FEA=mRNA /GEN=FGFR2 /PROD=fibroblast growth factor receptor 2, isoform 2precursor /DB_XREF=gi:13186252 /UG=Hs.278581 fibroblast growth factor receptor 2 (bacteria-expressed kinase, keratinocyte growth factor receptor, craniofacial dysostosis 1, Crouzon syndrome, Pfeiffer syndrome, Jackson-Weiss syndrome) /FL=gb:NM_022969.1 gb:M97193.1 gb:M80634.1	
mucin 3B	Consensus includes gb:AB038783.1 /DEF=Homo sapiens MUC3B mRNA for intestinal mucin, partial cds. /FEA=mRNA /GEN=MUC3B /PROD=intestinal mucin /DB_XREF=gi:9929917 /UG=Hs.129782 mucin 3A, intestinal	214898_x_at
AA	Consensus includes gb:AV728958 /FEA=EST /DB_XREF=gi:10838379 /DB_XREF=est:AV728958 /CLONE=HTCBYF04 /UG=Hs.150443 KIAA0320 protein	212703_at
CUG triplet repeat, RNA-binding protein 2	gb:NM_006561.1 /DEF=Homo sapiens CUG triplet repeat, RNA-binding protein 2 (CUGBP2), mRNA. /FEA=mRNA /GEN=CUGBP2 /PROD=CUG triplet repeat, RNA-binding protein 2 /DB_XREF=gi:5729815 /UG=Hs.211610 CUG triplet repeat, RNA-binding protein 2 /FL=gb:U69546.1 gb:AF036956.1 gb:AF090694.1 gb:NM_006561.1	202158_s_at
spondin 1, (f-spondin) extracellular matrix protein	gb:AB051390.1 /DEF=Homo sapiens mRNA for VSGPF-spondin, complete cds. /FEA=mRNA /PROD=VSGPF-spondin /DB_XREF=gi:11320819 /UG=Hs.5378 spondin 1, (f-spondin) extracellular matrix protein /FL=gb:AB051390.1	209437_s_at
mucin 3B	Consensus includes gb:AF113616 /DEF=Homo sapiens intestinal mucin 3 (MUC3) gene, partial cds /FEA=mRNA /DB_XREF=gi:6466800 /UG=Hs.129782 mucin 3A, intestinal	214676_x_at
EphA1	gb:NM_005232.1 /DEF=Homo sapiens EphA1 (EPHA1), mRNA. /FEA=mRNA /GEN=EPHA1 /PROD=EphA1 /DB_XREF=gi:4885208 /UG=Hs.89839 EphA1 /FL=gb:M18391.1 gb:NM_005232.1	205977_s_at
matrilin 3	gb:NM_002381.2 /DEF=Homo sapiens matrilin 3 (MATN3) precursor, mRNA. /FEA=mRNA /GEN=MATN3 /PROD=matrilin 3 precursor /DB_XREF=gi:13518040 /UG=Hs.278461	206091_at

	matrilin 3 /FL=gb:NM_002381.2	
bone morphogenetic protein 2	gb:NM_001200.1 /DEF=Homo sapiens bone morphogenetic protein 2 (BMP2), mRNA. /FEA=mRNA /GEN=BMP2 /PROD=bone morphogenetic protein 2 precursor /DB_XREF=gi:4557368 /UG=Hs.73853 bone morphogenetic protein 2 /FL=gb:NM_001200.1	205290_s_at
interferon consensus sequence binding protein 1	Consensus includes gb:AI073984 /FEA=EST /DB_XREF=gi:3400628 /DB_XREF=est:oy66c05.x1 /CLONE=IMAGE:1670792 /UG=Hs.14453 interferon consensus sequence binding protein 1 /FL=gb:M91196.1 gb:NM_002163.1	204057_at
retinoic acid receptor responder (tazarotene induced) 1	Consensus includes gb:AI669229 /FEA=EST /DB_XREF=gi:4834003 /DB_XREF=est:wc13e06.x1 /CLONE=IMAGE:2315074 /UG=Hs.82547 retinoic acid receptor responder (tazarotene induced) 1	221872_at
cystic fibrosis transmembrane conductance regulator, ATP-binding cassette (sub-family C, member 7)	Consensus includes gb:W60595 /FEA=EST /DB_XREF=gi:1367354 /DB_XREF=est:zc91b04.s1 /CLONE=IMAGE:338479 /UG=Hs.663 cystic fibrosis transmembrane conductance regulator, ATP-binding cassette (sub-family C, member 7)	215703_at
fibroblast growth factor receptor 2 (bacteria-expressed kinase, keratinocyte growth factor receptor, craniofacial dysostosis 1, Crouzon syndrome, Pfeiffer syndrome, Jackson-Weiss syndrome)	gb:M87771.1 /DEF=Human secreted fibroblast growth factor receptor (K-sam-III) mRNA, complete cds. /FEA=mRNA /GEN=K-sam-III /PROD=fibroblast growth factor receptor /DB_XREF=gi:186781 /UG=Hs.278581 fibroblast growth factor receptor 2 (bacteria-expressed kinase, keratinocyte growth factor receptor, craniofacial dysostosis 1, Crouzon syndrome, Pfeiffer syndrome, Jackson-Weiss syndrome) /FL=gb:NM_022970.1 gb:M87771.1	208228_s_at
myosin, heavy polypeptide 13, skeletal muscle	gb:NM_003802.1 /DEF=Homo sapiens myosin, heavy polypeptide 13, skeletal muscle (MYH13), mRNA. /FEA=mRNA /GEN=MYH13 /PROD=myosin, heavy polypeptide 13, skeletal muscle /DB_XREF=gi:11321578 /UG=Hs.278488 myosin, heavy polypeptide 13, skeletal muscle /FL=gb:NM_003802.1	208208_at

	gb:AF111782.2	
ESTs, Weakly similar to I38022 hypothetical protein [H.sapiens]	Consensus includes gb:AW675655 /FEA=EST /DB_XREF=gi:7540890 /DB_XREF=est:ba52e01.x1 /CLONE=IMAGE:2900184 /UG=Hs.314158 ESTs	222354_at
hypothetical protein FLJ20174	gb:NM_017699.1 /DEF=Homo sapiens hypothetical protein FLJ20174 (FLJ20174), mRNA. /FEA=mRNA /GEN=FLJ20174 /PROD=hypothetical protein FLJ20174 /DB_XREF=gi:8923170 /UG=Hs.114556 hypothetical protein FLJ20174 /FL=gb:NM_017699.1	219734_at
PTPRF interacting protein, binding protein 2 (liprin beta 2)	Consensus includes gb:AI692180 /FEA=EST /DB_XREF=gi:4969520 /DB_XREF=est:wd37f06.x1 /CLONE=IMAGE:2330339 /UG=Hs.12953 PTPRF interacting protein, binding protein 2 (liprin beta 2)	212841_s_at
ribonuclease, RNase A family, 1 (pancreatic)	gb:NM_002933.1 /DEF=Homo sapiens ribonuclease, RNase A family, 1 (pancreatic) (RNASE1), mRNA. /FEA=mRNA /GEN=RNASE1 /PROD=ribonuclease, RNase A family, 1 (pancreatic) /DB_XREF=gi:4506546 /UG=Hs.78224 ribonuclease, RNase A family, 1 (pancreatic) /FL=gb:BC005324.1 gb:NM_002933.1 gb:D26129.1	201785_at
hairless (mouse) homolog	gb:NM_018411.1 /DEF=Homo sapiens hairless protein (putative single zinc finger transcription factor protein, responsible for autosomal recessive universal congenital alopecia, HR gene) (HSA277165), mRNA. /FEA=mRNA /GEN=HSA277165 /PROD=hairless protein /DB_XREF=gi:11036651 /UG=Hs.272367 hairless protein (putative single zinc finger transcription factor protein, responsible for autosomal recessive universal congenital alopecia, HR gene) /FL=gb:NM_018411.1	220163_s_at
nuclear receptor subfamily 5, group A, member 2	Consensus includes gb:AF228413.1 /DEF=Homo sapiens hepatocyte transcription factor mRNA, 3UTR. /FEA=mRNA /DB_XREF=gi:7677372 /UG=Hs.183123 nuclear receptor subfamily 5, group A, member 2 /FL=gb:U93553.1 gb:AB019246.1 gb:AF124247.1	210174_at
superoxide dismutase 3, extracellular	gb:NM_003102.1 /DEF=Homo sapiens superoxide dismutase 3, extracellular (SOD3), mRNA. /FEA=mRNA /GEN=SOD3 /PROD=superoxide dismutase 3, extracellular	205236_x_at

	/DB_XREF=gi:4507150 /UG=Hs.2420 superoxide dismutase 3, extracellular /FL=gb:J02947.1 gb:NM_003102.1	
zinc finger protein 137 (clone pHZ-30)	gb:NM_003438.1 /DEF=Homo sapiens zinc finger protein 137 (clone pHZ-30) (ZNF137), mRNA. /FEA=mRNA /GEN=ZNF137 /PROD=zinc finger protein 137 (clone pHZ-30) /DB_XREF=gi:4507988 /UG=Hs.151689 zinc finger protein 137 (clone pHZ-30) /FL=gb:NM_003438.1 gb:U09414.1	207394_at
Homo sapiens mRNA; cDNA DKFZp564D042 (from clone DKFZp564D042)	Consensus includes gb:AL049983.1 /DEF=Homo sapiens mRNA; cDNA DKFZp564D042 (from clone DKFZp564D042). /FEA=mRNA /DB_XREF=gi:4884234 /UG=Hs.240136 Homo sapiens mRNA; cDNA DKFZp564D042 (from clone DKFZp564D042)	217288_at
Hermansky-Pudlak syndrome	Consensus includes gb:AL022313 /DEF=Human DNA sequence from clone RP5-1119A7 on chromosome 22q12.2-12.3 Contains the TXN2 gene for mitochondrial thioredoxin, a novel gene, the EIF3S7 gene for eukaryotic translation initiation factor 3 subunit 7 (zeta, 6667kD) (EIF3-P66), the gene f... /FEA=CDS_3 /DB_XREF=gi:4200326 /UG=Hs.272270 Human DNA sequence from clone RP5-1119A7 on chromosome 22q12.2-12.3 Contains the TXN2 gene for mitochondrial thioredoxin, a novel gene, the EIF3S7 gene for eukaryotic translation initiation factor 3 subunit 7 (zeta, 6667kD) (EIF3-P66), the gene for a nov	217354_s_at
peroxisomal trans 2-enoyl CoA reductase; putative short chain alcohol dehydrogenase	gb:NM_018441.1 /DEF=Homo sapiens peroxisomal trans 2-enoyl CoA reductase; putative short chain alcohol dehydrogenase (HSA250303), mRNA. /FEA=mRNA /GEN=HSA250303 /PROD=peroxisomal trans 2-enoyl CoA reductase;putative short chain alcohol dehydrogenase /DB_XREF=gi:8923751 /UG=Hs.281680 peroxisomal trans 2-enoyl CoA reductase; putative short chain alcohol dehydrogenase /FL=gb:NM_018441.1	221142_s_at
BTG family, member 2	gb:NM_006763.1 /DEF=Homo sapiens BTG family, member 2 (BTG2), mRNA. /FEA=mRNA /GEN=BTG2 /PROD=BTG family, member 2 /DB_XREF=gi:5802987 /UG=Hs.75462 BTG family, member 2 /FL=gb:U72649.1 gb:NM_006763.1	201236_s_at
phosducin	gb:M33478.1 /DEF=Human 33-kDa phototransducing protein mRNA, complete cds.	211496_s_at

	/FEA=mRNA /DB_XREF=gi:177186 /UG=Hs.550 phosducin /FL=gb:NM_022577.1 gb:M33478.1 gb:AF076465.1	
Rho GTPase activating protein 8	gb:NM_015366.1 /DEF=Homo sapiens Rho GTPase activating protein 8 (ARHGAP8), mRNA. /FEA=mRNA /GEN=ARHGAP8 /PROD=Rho GTPase activating protein 8 /DB_XREF=gi:7656903 /UG=Hs.102336 Rho GTPase activating protein 8 /FL=gb:NM_015366.1	205980_s_at
Homo sapiens clone 24707 mRNA sequence	Consensus includes gb:AW593996 /FEA=EST /DB_XREF=gi:7281254 /DB_XREF=est:hg41g06.x1 /CLONE=IMAGE:2948218 /UG=Hs.124969 Homo sapiens clone 24707 mRNA sequence	213256_at
caspase 10, apoptosis-related cysteine protease	gb:NM_001230.1 /DEF=Homo sapiens caspase 10, apoptosis-related cysteine protease (CASP10), mRNA. /FEA=mRNA /GEN=CASP10 /PROD=caspase 10, apoptosis-related cysteine protease /DB_XREF=gi:4502568 /UG=Hs.5353 caspase 10, apoptosis-related cysteine protease /FL=gb:U60519.1 gb:NM_001230.1	205467_at
KIAA0690 protein	Consensus includes gb:AK000238.1 /DEF=Homo sapiens cDNA FLJ20231 fis, clone COLF5511, highly similar to AB014590 Homo sapiens mRNA for KIAA0690 protein. /FEA=mRNA /DB_XREF=gi:7020188 /UG=Hs.60103 KIAA0690 protein	216360_x_at
Homo sapiens, Similar to RIKEN cDNA 1810037C20 gene, clone MGC:21481 IMAGE:3852062, mRNA, complete cds	Consensus includes gb:AW001287 /FEA=EST /DB_XREF=gi:5848203 /DB_XREF=est:wu27e06.x1 /CLONE=IMAGE:2521282 /UG=Hs.61265 ESTs, Weakly similar to G786_HUMAN PROTEIN GS3786 H.sapiens	227676_at
ESTs	Consensus includes gb:AA581439 /FEA=EST /DB_XREF=gi:2359211 /DB_XREF=est:nh13c10.s1 /CLONE=IMAGE:952242 /UG=Hs.152328 ESTs	244650_at
ESTs	Consensus includes gb:AI739241 /FEA=EST /DB_XREF=gi:5101222 /DB_XREF=est:wi14h02.x1 /CLONE=IMAGE:2390259 /UG=Hs.171480 ESTs	238984_at

hypothetical protein FLJ23045	Consensus includes gb:AB046810.1 /DEF=Homo sapiens mRNA for KIAA1590 protein, partial cds. /FEA=mRNA /GEN=KIAA1590 /PROD=KIAA1590 protein /DB_XREF=gi:10047254 /UG=Hs.101774 hypothetical protein FLJ23045	232083_at
regenerating gene type IV	gb:AY007243.1 /DEF=Homo sapiens regenerating gene type IV mRNA, complete cds. /FEA=mRNA /PROD=regenerating gene type IV /DB_XREF=gi:12621025 /UG=Hs.105484 Homo sapiens regenerating gene type IV mRNA, complete cds /FL=gb:AY007243.1	223447_at
ESTs	Consensus includes gb:AI139990 /FEA=EST /DB_XREF=gi:3647447 /DB_XREF=est:qa47d03.x1 /CLONE=IMAGE:1689893 /UG=Hs.134586 ESTs	231022_at
ESTs	Consensus includes gb:AI733801 /FEA=EST /DB_XREF=gi:5054914 /DB_XREF=est:qk39c04.x5 /CLONE=IMAGE:1871334 /UG=Hs.146186 ESTs	237923_at
hypothetical protein MGC20702	Consensus includes gb:AK002203.1 /DEF=Homo sapiens cDNA FLJ11341 fis, clone PLACE1010786. /FEA=mRNA /DB_XREF=gi:7023932 /UG=Hs.10260 Homo sapiens cDNA FLJ11341 fis, clone PLACE1010786	226992_at
ESTs, Weakly similar to ALU1_HUMAN ALU SUBFAMILY J SEQUENCE CONTAMINATION WARNING ENTRY [H.sapiens]	Consensus includes gb:AI457984 /FEA=EST /DB_XREF=gi:4312002 /DB_XREF=est:tj66a04.x1 /CLONE=IMAGE:2146446 /UG=Hs.165900 ESTs, Weakly similar to ALUC_HUMAN !!!! ALU CLASS C WARNING ENTRY !!! H.sapiens	243729_at
Homo sapiens cDNA: FLJ22063 fis, clone HEP10326	Consensus includes gb:T86159 /FEA=EST /DB_XREF=gi:714511 /DB_XREF=est:yd84h07.s1 /CLONE=IMAGE:114973 /UG=Hs.10450 Homo sapiens cDNA: FLJ22063 fis, clone HEP10326	227724_at
ESTs	Consensus includes gb:AI806131 /FEA=EST /DB_XREF=gi:5392697 /DB_XREF=est:wf06c06.x1 /CLONE=IMAGE:2349802 /UG=Hs.99376	231148_at

	ESTs	
anterior gradient 2 (Xenepus laevis) homolog	Consensus includes gb:AI922323 /FEA=EST /DB_XREF=gi:5658287 /DB_XREF=est:wn90h03.x1 /CLONE=IMAGE:2453141 /UG=Hs.293380 ESTs	228969_at
ESTs	Consensus includes gb:AI493909 /FEA=EST /DB_XREF=gi:4394912 /DB_XREF=est:qz94e02.x1 /CLONE=IMAGE:2042234 /UG=Hs.6131 ESTs	235562_at
hypothetical protein FLJ22233	Consensus includes gb:AI339568 /FEA=EST /DB_XREF=gi:4076495 /DB_XREF=est:qk67e10.x1 /CLONE=IMAGE:1874058 /UG=Hs.286194 hypothetical protein FLJ22233 /FL=gb:NM_024959.1	222727_s_at
GalNAc alpha-2, 6-sialyltransferase I, long form	Consensus includes gb:Y11339.2 /DEF=Homo sapiens mRNA for GalNAc alpha-2, 6-sialyltransferase I, long form. /FEA=mRNA /PROD=GalNAc alpha-2,6-sialyltransferase I /DB_XREF=gi:7576275 /UG=Hs.105352 GalNAc alpha-2, 6-sialyltransferase I, long form	227725_at
ESTs	Consensus includes gb:AI917390 /FEA=EST /DB_XREF=gi:5637245 /DB_XREF=est:ts79a05.x1 /CLONE=IMAGE:2237456 /UG=Hs.99415 ESTs	240964_at
Homo sapiens cDNA: FLJ22751 fis, clone KAIA0483, highly similar to AF016692 Homo sapiens small intestinal mucin (MUC3) mRNA	Consensus includes gb:AK026404.1 /DEF=Homo sapiens cDNA: FLJ22751 fis, clone KAIA0483, highly similar to AF016692 Homo sapiens small intestinal mucin (MUC3) mRNA. /FEA=mRNA /DB_XREF=gi:10439257 /UG=Hs.271819 Homo sapiens cDNA: FLJ22751 fis, clone KAIA0483, highly similar to AF016692 Homo sapiens small intestinal mucin (MUC3) mRNA	232321_at
Homo sapiens cDNA: FLJ23331 fis, clone HEP12664	Consensus includes gb:AK026984.1 /DEF=Homo sapiens cDNA: FLJ23331 fis, clone HEP12664. /FEA=mRNA /DB_XREF=gi:10439980 /UG=Hs.50742 Homo sapiens cDNA: FLJ23331 fis, clone HEP12664	229021_at
ESTs	Consensus includes gb:AA827649 /FEA=EST /DB_XREF=gi:2900090 /DB_XREF=est:od01a12.s1 /CLONE=IMAGE:1357918 /UG=Hs.105317 ESTs	235515_at
prostate cancer	Consensus includes gb:AA633076 /FEA=EST	226167_at

associated protein 7	/DB_XREF=gi:2556490 /DB_XREF=est:nq38a06.s1 /CLONE=IMAGE:1146130 /UG=Hs.27495 prostate cancer associated protein 7	
ESTs	Consensus includes gb:N37023 /FEA=EST /DB_XREF=gi:1158165 /DB_XREF=est:yy40d03.s1 /CLONE=IMAGE:273701 /UG=Hs.235883 ESTs	225407_at
ESTs, Weakly similar to I38588 reverse transcriptase homolog [H.sapiens]	Consensus includes gb:AI864053 /FEA=EST /DB_XREF=gi:5528160 /DB_XREF=est:wj55h10.x1 /CLONE=IMAGE:2406787 /UG=Hs.39972 ESTs, Weakly similar to I38588 reverse transcriptase homolog H.sapiens	235678_at
ESTs, Weakly similar to JX0331 laurate omega-hydroxylase [H.sapiens]	Consensus includes gb:AA557324 /FEA=EST /DB_XREF=gi:2327801 /DB_XREF=est:nl81a02.s1 /CLONE=IMAGE:1057034 /UG=Hs.26040 ESTs, Weakly similar to fatty acid omega-hydroxylase H.sapiens	227702_at
ESTs	Consensus includes gb:BF594323 /FEA=EST /DB_XREF=gi:11686647 /DB_XREF=est:7h79g07.x1 /CLONE=IMAGE:3322236 /UG=Hs.158989 ESTs	238103_at
ESTs, Weakly similar to JE0350 Anterior gradient-2 [H.sapiens]	Consensus includes gb:AI827789 /FEA=EST /DB_XREF=gi:5448449 /DB_XREF=est:wf33a07.x1 /CLONE=IMAGE:2357364 /UG=Hs.100686 ESTs, Weakly similar to JE0350 Anterior gradient-2 H.sapiens	228241_at
ESTs	Consensus includes gb:AI968097 /FEA=EST /DB_XREF=gi:5764915 /DB_XREF=est:wu13a12.x1 /CLONE=IMAGE:2516830 /UG=Hs.131360 ESTs	237835_at
ESTs	Consensus includes gb:H05025 /FEA=EST /DB_XREF=gi:868577 /DB_XREF=est:yl74g12.s1 /CLONE=IMAGE:43864 /UG=Hs.323767 ESTs	241874_at
Homo sapiens, Similar to RIKEN cDNA 1110060O18 gene, clone MGC:17236 IMAGE:386413	Consensus includes gb:AA524690 /FEA=EST /DB_XREF=gi:2265618 /DB_XREF=est:ng38e07.s1 /CLONE=IMAGE:937092 /UG=Hs.294143 ESTs, Weakly similar to predicted using Genefinder C.elegans	226168_at

7, mRNA, complete cds		
ESTs	Consensus includes gb:AI300126 /FEA=EST /DB_XREF=gi:3959472 /DB_XREF=est:qn54f02.x1 /CLONE=IMAGE:1902075 /UG=Hs.257858 ESTs	240830_at
Homo sapiens cDNA FLJ13137 fis, clone NT2RP3003150	Consensus includes gb:AA129774 /FEA=EST /DB_XREF=gi:1690185 /DB_XREF=est:zl16h09.s1 /CLONE=IMAGE:502145 /UG=Hs.288905 Homo sapiens cDNA FLJ13137 fis, clone NT2RP3003150	227019_at
ESTs	Consensus includes gb:AW024656 /FEA=EST /DB_XREF=gi:5878186 /DB_XREF=est:wu78h05.x1 /CLONE=IMAGE:2526201 /UG=Hs.233382 ESTs, Moderately similar to AF119917 62 PRO2822 H.sapiens	242358_at

The biomarker probe set list B (Table 3) contains 95 probe sets (U133A: 47; U133B 48). The biomarker probe set list B contains polynucleotides identified to be biomarkers of EGFR antagonist sensitivity employing strategy B. In strategy B, polynucleotides were required to satisfy a stringent criteria for correlation to IC_{50} values and a less stringent condition for EGFR status coregulation. Namely, the polynucleotides had to have a Pearsons correlation of -0.5 or less with respect to IC_{50} and be called absent by the Affymetrix software in 5 out of the 6 cell lines with lowest expression of EGFR.

TABLE 3 - Biomarker Probe Set List B

Unigene Title	Affymetrix Description	Affymetrix probe set
dopa decarboxylase (aromatic L-amino acid decarboxylase)	Consensus includes gb:AW772056 /FEA=EST /DB_XREF=gi:7704118 /DB_XREF=est:hn64g06.x1 /CLONE=IMAGE:3032698 /UG=Hs.150403 dopa decarboxylase (aromatic L-amino acid decarboxylase)	214347_s_at
cystic fibrosis transmembrane conductance regulator, ATP-binding cassette	gb:NM_000492.2 /DEF=Homo sapiens cystic fibrosis transmembrane conductance regulator, ATP-binding cassette (sub-family C, member 7) (CFTR), mRNA. /FEA=mRNA /GEN=CFTR /PROD=cystic fibrosis transmembrane	205043_at

(sub-family C, member 7)	conductanceregulator, ATP-binding cassette (sub-family C, member 7) /DB_XREF=gi:6995995 /UG=Hs.663 cystic fibrosis transmembrane conductance regulator, ATP-binding cassette (sub-family C, member 7) /FL=gb:NM_000492.2	
carcinoembryonic antigen-related cell adhesion molecule 6 (non-specific cross reacting antigen)	gb:BC005008.1 /DEF=Homo sapiens, carcinoembryonic antigen-related cell adhesion molecule 6 (non-specific cross reacting antigen), clone MGC:10467, mRNA, complete cds. /FEA=mRNA /PROD=carcinoembryonic antigen-related cell adhesionmolecule 6 (non-specific cross reacting antigen) /DB_XREF=gi:13477106 /UG=Hs.73848 carcinoembryonic antigen-related cell adhesion molecule 6 (non-specific cross reacting antigen) /FL=gb:BC005008.1 gb:M18216.1 gb:M29541.1 gb:NM_002483.1	203757_s_at
hypothetical protein FLJ20075	gb:NM_017655.1 /DEF=Homo sapiens hypothetical protein FLJ20075 (FLJ20075), mRNA. /FEA=mRNA /GEN=FLJ20075 /PROD=hypothetical protein FLJ20075 /DB_XREF=gi:8923083 /UG=Hs.205058 hypothetical protein FLJ20075 /FL=gb:NM_017655.1	219970_at
ATPase, Class V, type 10B	Consensus includes gb:AW006935 /FEA=EST /DB_XREF=gi:5855713 /DB_XREF=est:wt08b11.x1 /CLONE=IMAGE:2506845 /UG=Hs.109358 ATPase, Class V, type 10B	214070_s_at
cystic fibrosis transmembrane conductance regulator, ATP-binding cassette (sub-family C, member 7)	Consensus includes gb:W60595 /FEA=EST /DB_XREF=gi:1367354 /DB_XREF=est:zc91b04.s1 /CLONE=IMAGE:338479 /UG=Hs.663 cystic fibrosis transmembrane conductance regulator, ATP-binding cassette (sub-family C, member 7)	215702_s_at
HERV-H LTR-associating 2	gb:NM_007072.1 /DEF=Homo sapiens HERV-H LTR-associating 2 (HHLA2), mRNA. /FEA=mRNA /GEN=HHLA2 /PROD=HERV-H LTR-associating 2 /DB_XREF=gi:5901963 /UG=Hs.252351 HERV-H LTR-associating 2 /FL=gb:AF126162.1 gb:NM_007072.1	220812_s_at
AA	Consensus includes gb:AV728958 /FEA=EST /DB_XREF=gi:10838379 /DB_XREF=est:AV728958 /CLONE=HTCBYF04 /UG=Hs.150443 KIAA0320 protein	212703_at

hemoglobin, alpha 2	Consensus includes gb:T50399 /FEA=EST /DB_XREF=gi:652259 /DB_XREF=est:yb30b11.s1 /CLONE=IMAGE:72669 /UG=Hs.251577 hemoglobin, alpha 1	214414_x_at
spondin 1, (f-spondin) extracellular matrix protein	Consensus includes gb:AI885290 /FEA=EST /DB_XREF=gi:5590454 /DB_XREF=est:wl92a04.x1 /CLONE=IMAGE:2432334 /UG=Hs.5378 spondin 1, (f-spondin) extracellular matrix protein	213993_at
hemoglobin, alpha 1	gb:BC005931.1 /DEF=Homo sapiens, hemoglobin, alpha 2, clone MGC:14541, mRNA, complete cds. /FEA=mRNA /PROD=hemoglobin, alpha 2 /DB_XREF=gi:13543547 /FL=gb:BC005931.1	211745_x_at
serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 5	gb:NM_002639.1 /DEF=Homo sapiens serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 5 (SERPINB5), mRNA. /FEA=mRNA /GEN=SERPINB5 /PROD=serine (or cysteine) proteinase inhibitor, cladeB (ovalbumin), member 5 /DB_XREF=gi:4505788 /UG=Hs.55279 serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 5 /FL=gb:NM_002639.1 gb:U04313.1	204855_at
3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2 (mitochondrial)	gb:NM_005518.1 /DEF=Homo sapiens 3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2 (mitochondrial) (HMGCS2), mRNA. /FEA=mRNA /GEN=HMGCS2 /PROD=3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2(mitochondrial) /DB_XREF=gi:5031750 /UG=Hs.59889 3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2 (mitochondrial) /FL=gb:NM_005518.1	204607_at
anterior gradient 2 (Xenepus laevis) homolog	gb:AF088867.1 /DEF=Homo sapiens putative secreted protein XAG mRNA, complete cds. /FEA=mRNA /PROD=putative secreted protein XAG /DB_XREF=gi:6652811 /UG=Hs.91011 anterior gradient 2 (Xenepus laevis) homolog /FL=gb:AF007791.1 gb:AF038451.1 gb:NM_006408.1 gb:AF088867.1	209173_at
FXYD domain-containing ion transport regulator 3	gb:BC005238.1 /DEF=Homo sapiens, FXYD domain-containing ion transport regulator 3, clone MGC:12265, mRNA, complete cds. /FEA=mRNA /PROD=FXYD domain-containing ion transport regulator3 /DB_XREF=gi:13528881 /UG=Hs.301350 FXYD domain-containing ion transport regulator	202489_s_at

	3 /FL=gb:NM_005971.2 gb:BC005238.1	
dipeptidylpeptidase IV (CD26, adenosine deaminase complexing protein 2)	gb:M80536.1 /DEF=H.sapiens dipeptidyl peptidase IV (DPP4) mRNA, complete cds. /FEA=mRNA /GEN=DPP4 /PROD=dipeptidyl peptidase IV /DB_XREF=gi:181569 /UG=Hs.44926 dipeptidylpeptidase IV (CD26, adenosine deaminase complexing protein 2) /FL=gb:M80536.1 gb:NM_001935.1	203716_s_at
cystic fibrosis transmembrane conductance regulator, ATP-binding cassette (sub-family C, member 7)	Consensus includes gb:W60595 /FEA=EST /DB_XREF=gi:1367354 /DB_XREF=est:zc91b04.s1 /CLONE=IMAGE:338479 /UG=Hs.663 cystic fibrosis transmembrane conductance regulator, ATP-binding cassette (sub-family C, member 7)	215703_at
EphA1	gb:NM_005232.1 /DEF=Homo sapiens EphA1 (EPHA1), mRNA. /FEA=mRNA /GEN=EPHA1 /PROD=EphA1 /DB_XREF=gi:4885208 /UG=Hs.89839 EphA1 /FL=gb:M18391.1 gb:NM_005232.1	205977_s_at
spondin 1, (f-spondin) extracellular matrix protein	Consensus includes gb:AI885290 /FEA=EST /DB_XREF=gi:5590454 /DB_XREF=est:wl92a04.x1 /CLONE=IMAGE:2432334 /UG=Hs.5378 spondin 1, (f-spondin) extracellular matrix protein	213994_s_at
CUG triplet repeat, RNA-binding protein 2	gb:NM_006561.1 /DEF=Homo sapiens CUG triplet repeat, RNA-binding protein 2 (CUGBP2), mRNA. /FEA=mRNA /GEN=CUGBP2 /PROD=CUG triplet repeat, RNA-binding protein 2 /DB_XREF=gi:5729815 /UG=Hs.211610 CUG triplet repeat, RNA-binding protein 2 /FL=gb:U69546.1 gb:AF036956.1 gb:AF090694.1 gb:NM_006561.1	202158_s_at
DKFZP434C091 protein	Consensus includes gb:AL080170.1 /DEF=Homo sapiens mRNA; cDNA DKFZp434C091 (from clone DKFZp434C091); partial cds. /FEA=mRNA /GEN=DKFZp434C091 /PROD=hypothetical protein /DB_XREF=gi:5262639 /UG=Hs.51692 DKFZP434C091 protein	215047_at
mucin 3B	Consensus includes gb:AF113616 /DEF=Homo sapiens intestinal mucin 3 (MUC3) gene, partial cds /FEA=mRNA /DB_XREF=gi:6466800 /UG=Hs.129782 mucin 3A, intestinal	214676_x_at
potassium channel,	gb:U90065.1 /DEF=Human potassium channel KCNO1 mRNA, complete cds. /FEA=mRNA	204678_s_at

subfamily K, member 1 (TWIK-1)	/PROD=potassium channel KCNO1 /DB_XREF=gi:1916294 /UG=Hs.79351 potassium channel, subfamily K, member 1 (TWIK-1) /FL=gb:U33632.1 gb:U90065.1 gb:U76996.1 gb:NM_002245.1	
nuclear receptor subfamily 3, group C, member 2	gb:NM_000901.1 /DEF=Homo sapiens nuclear receptor subfamily 3, group C, member 2 (NR3C2), mRNA. /FEA=mRNA /GEN=NR3C2 /PROD=nuclear receptor subfamily 3, group C, member 2 /DB_XREF=gi:4505198 /UG=Hs.1790 nuclear receptor subfamily 3, group C, member 2 /FL=gb:M16801.1 gb:NM_000901.1	205259_at
BTG family, member 2	gb:NM_006763.1 /DEF=Homo sapiens BTG family, member 2 (BTG2), mRNA. /FEA=mRNA /GEN=BTG2 /PROD=BTG family, member 2 /DB_XREF=gi:5802987 /UG=Hs.75462 BTG family, member 2 /FL=gb:U72649.1 gb:NM_006763.1	201236_s_at
G protein-coupled receptor 49	gb:AF062006.1 /DEF=Homo sapiens orphan G protein-coupled receptor HG38 mRNA, complete cds. /FEA=mRNA /PROD=orphan G protein-coupled receptor HG38 /DB_XREF=gi:3366801 /UG=Hs.285529 G protein-coupled receptor 49 /FL=gb:AF062006.1 gb:AF061444.1 gb:NM_003667.1	210393_at
hypothetical protein FLJ20048	gb:NM_017640.1 /DEF=Homo sapiens hypothetical protein FLJ20048 (FLJ20048), mRNA. /FEA=mRNA /GEN=FLJ20048 /PROD=hypothetical protein FLJ20048 /DB_XREF=gi:8923056 /UG=Hs.116470 hypothetical protein FLJ20048 /FL=gb:NM_017640.1	219573_at
cytochrome P450, subfamily IIJ (arachidonic acid epoxygenase) polypeptide 2	gb:NM_000775.1 /DEF=Homo sapiens cytochrome P450, subfamily IIJ (arachidonic acid epoxygenase) polypeptide 2 (CYP2J2), mRNA. /FEA=mRNA /GEN=CYP2J2 /PROD=cytochrome P450, subfamily IIJ (arachidonic acidepoxygenase) polypeptide 2 /DB_XREF=gi:4503226 /UG=Hs.152096 cytochrome P450, subfamily IIJ (arachidonic acid epoxygenase) polypeptide 2 /FL=gb:U37143.1 gb:NM_000775.1	205073_at
brain-specific protein p25 alpha	gb:NM_007030.1 /DEF=Homo sapiens brain-specific protein p25 alpha (p25), mRNA. /FEA=mRNA /GEN=p25 /PROD=brain-specific protein p25 alpha /DB_XREF=gi:5902017 /UG=Hs.29353 brain-specific protein p25 alpha	206179_s_at

	/FL=gb:AB017016.1 gb:NM_007030.1	
mucin 2, intestinal/trachea l	gb:NM_002457.1 /DEF=Homo sapiens mucin 2, intestinaltracheal (MUC2), mRNA. /FEA=mRNA /GEN=MUC2 /PROD=mucin 2, intestinaltracheal /DB_XREF=gi:4505284 /UG=Hs.315 mucin 2, intestinaltracheal /FL=gb:NM_002457.1 gb:L21998.1	204673_at
hypothetical protein FLJ20174	gb:NM_017699.1 /DEF=Homo sapiens hypothetical protein FLJ20174 (FLJ20174), mRNA. /FEA=mRNA /GEN=FLJ20174 /PROD=hypothetical protein FLJ20174 /DB_XREF=gi:8923170 /UG=Hs.114556 hypothetical protein FLJ20174 /FL=gb:NM_017699.1	219734_at
metastasis-associated 1-like 1	gb:NM_004739.1 /DEF=Homo sapiens metastasis-associated 1-like 1 (MTA1L1), mRNA. /FEA=mRNA /GEN=MTA1L1 /PROD=metastasis-associated 1-like 1 /DB_XREF=gi:4758739 /UG=Hs.173043 metastasis-associated 1-like 1 /FL=gb:AB016591.1 gb:NM_004739.1 gb:AF295807.1	203444_s_at
bone morphogenetic protein 2	gb:NM_001200.1 /DEF=Homo sapiens bone morphogenetic protein 2 (BMP2), mRNA. /FEA=mRNA /GEN=BMP2 /PROD=bone morphogenetic protein 2 precursor /DB_XREF=gi:4557368 /UG=Hs.73853 bone morphogenetic protein 2 /FL=gb:NM_001200.1	205290_s_at
heparanase	gb:NM_006665.1 /DEF=Homo sapiens heparanase (HPSE), mRNA. /FEA=mRNA /GEN=HPSE /PROD=heparanase /DB_XREF=gi:5729872 /UG=Hs.44227 heparanase /FL=gb:AF165154.1 gb:AF152376.1 gb:NM_006665.1 gb:AF084467.1 gb:AF155510.1	219403_s_at
tumor necrosis factor receptor superfamily, member 14 (herpesvirus entry mediator)	gb:BC002794.1 /DEF=Homo sapiens, tumor necrosis factor receptor superfamily, member 14 (herpesvirus entry mediator), clone MGC:3753, mRNA, complete cds. /FEA=mRNA /PROD=tumor necrosis factor receptor superfamily,member 14 (herpesvirus entry mediator) /DB_XREF=gi:12803894 /UG=Hs.279899 tumor necrosis factor receptor superfamily, member 14 (herpesvirus entry mediator) /FL=gb:BC002794.1 gb:U70321.1 gb:U81232.1 gb:NM_003820.1 gb:AF153978.1	209354_at
CUG triplet repeat, RNA-	Consensus includes gb:N36839 /FEA=EST /DB_XREF=gi:1157981	202156_s_at

binding protein 2	/DB_XREF=est:yy35f07.s1 /CLONE=IMAGE:273253 /UG=Hs.211610 CUG triplet repeat, RNA-binding protein 2 /FL=gb:U69546.1 gb:AF036956.1 gb:AF090694.1 gb:NM_006561.1	
ESTs, Moderately similar to AF078844 1 hqp0376 protein [H.sapiens]	Consensus includes gb:R06655 /FEA=EST /DB_XREF=gi:757275 /DB_XREF=est:yf10e02.r1 /CLONE=IMAGE:126458 /UG=Hs.188518 ESTs, Moderately similar to AF078844 1 hqp0376 protein H.sapiens	217546_at
hairless (mouse) homolog	gb:NM_018411.1 /DEF=Homo sapiens hairless protein (putative single zinc finger transcription factor protein, responsible for autosomal recessive universal congenital alopecia, HR gene) (HSA277165), mRNA. /FEA=mRNA /GEN=HSA277165 /PROD=hairless protein /DB_XREF=gi:11036651 /UG=Hs.272367 hairless protein (putative single zinc finger transcription factor protein, responsible for autosomal recessive universal congenital alopecia, HR gene) /FL=gb:NM_018411.1	220163_s_at
branched chain aminotransferase 1, cytosolic	Consensus includes gb:NM_005504.1 /DEF=Homo sapiens branched chain aminotransferase 1, cytosolic (BCAT1), mRNA. /FEA=CDS /GEN=BCAT1 /PROD=branched chain aminotransferase 1, cytosolic /DB_XREF=gi:5031606 /UG=Hs.157205 branched chain aminotransferase 1, cytosolic /FL=gb:U21551.1 gb:NM_005504.1	214452_at
pancreas-enriched phospholipase C	gb:NM_016341.1 /DEF=Homo sapiens pancreas-enriched phospholipase C (LOC51196), mRNA. /FEA=mRNA /GEN=LOC51196 /PROD=pancreas-enriched phospholipase C /DB_XREF=gi:7705940 /UG=Hs.6733 pancreas-enriched phospholipase C /FL=gb:AF190642.2 gb:AF117948.1 gb:NM_016341.1	205112_at
prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)	gb:NM_000963.1 /DEF=Homo sapiens prostaglandin-endoperoxide synthase 2 (prostaglandin GH synthase and cyclooxygenase) (PTGS2), mRNA. /FEA=mRNA /GEN=PTGS2 /PROD=prostaglandin-endoperoxide synthase 2(prostaglandin GH synthase and cyclooxygenase) /DB_XREF=gi:4506264 /UG=Hs.196384 prostaglandin-endoperoxide synthase 2 (prostaglandin GH synthase and	204748_at

	cyclooxygenase) /FL=gb:M90100.1 gb:L15326.1 gb:NM_000963.1	
phosphatase and tensin homolog (mutated in multiple advanced cancers 1)	gb:NM_000314.1 /DEF=Homo sapiens phosphatase and tensin homolog (mutated in multiple advanced cancers 1) (PTEN), mRNA. /FEA=mRNA /GEN=PTEN /PROD=phosphatase and tensin homolog (mutated inmultiple advanced cancers 1) /DB_XREF=gi:4506248 /UG=Hs.10712 phosphatase and tensin homolog (mutated in multiple advanced cancers 1) /FL=gb:U92436.1 gb:U93051.1 gb:U96180.1 gb:NM_000314.1	204054_at
retinoic acid receptor responder (tazarotene induced) 1	Consensus includes gb:AI669229 /FEA=EST /DB_XREF=gi:4834003 /DB_XREF=est:wc13e06.x1 /CLONE=IMAGE:2315074 /UG=Hs.82547 retinoic acid receptor responder (tazarotene induced) 1	221872_at
protease inhibitor 3, skin-derived (SKALP)	gb:NM_002638.1 /DEF=Homo sapiens protease inhibitor 3, skin-derived (SKALP) (PI3), mRNA. /FEA=mRNA /GEN=PI3 /PROD=protease inhibitor 3, skin-derived (SKALP) /DB_XREF=gi:4505786 /UG=Hs.112341 protease inhibitor 3, skin-derived (SKALP) /FL=gb:NM_002638.1	203691_at
zinc finger protein 137 (clone pHZ-30)	gb:NM_003438.1 /DEF=Homo sapiens zinc finger protein 137 (clone pHZ-30) (ZNF137), mRNA. /FEA=mRNA /GEN=ZNF137 /PROD=zinc finger protein 137 (clone pHZ-30) /DB_XREF=gi:4507988 /UG=Hs.151689 zinc finger protein 137 (clone pHZ-30) /FL=gb:NM_003438.1 gb:U09414.1	207394_at
myosin, light polypeptide 5, regulatory	gb:NM_002477.1 /DEF=Homo sapiens myosin, light polypeptide 5, regulatory (MYL5), mRNA. /FEA=mRNA /GEN=MYL5 /PROD=myosin, light polypeptide 5, regulatory /DB_XREF=gi:4505304 /UG=Hs.170482 myosin, light polypeptide 5, regulatory /FL=gb:L03785.1 gb:NM_002477.1	205145_s_at
tumor necrosis factor receptor superfamily, member 6	gb:NM_000043.1 /DEF=Homo sapiens tumor necrosis factor receptor superfamily, member 6 (TNFRSF6), mRNA. /FEA=mRNA /GEN=TNFRSF6 /PROD=apoptosis (APO-1) antigen 1 /DB_XREF=gi:4507582 /UG=Hs.82359 tumor necrosis factor receptor superfamily, member 6 /FL=gb:M67454.1 gb:NM_000043.1	204781_s_at
hypothetical	Consensus includes gb:AI339568 /FEA=EST	222727_s_at

protein FLJ22233	/DB_XREF=gi:4076495 /DB_XREF=est:qk67e10.x1 /CLONE=IMAGE:1874058 /UG=Hs.286194 hypothetical protein FLJ22233 /FL=gb:NM_024959.1	
regenerating gene type IV	gb:AY007243.1 /DEF=Homo sapiens regenerating gene type IV mRNA, complete cds. /FEA=mRNA /PROD=regenerating gene type IV /DB_XREF=gi:12621025 /UG=Hs.105484 Homo sapiens regenerating gene type IV mRNA, complete cds /FL=gb:AY007243.1	223447_at
Homo sapiens cDNA: FLJ21962 fis, clone HEP05564	Consensus includes gb:AK025615.1 /DEF=Homo sapiens cDNA: FLJ21962 fis, clone HEP05564. /FEA=mRNA /DB_XREF=gi:10438186 /UG=Hs.7567 Homo sapiens cDNA: FLJ21962 fis, clone HEP05564	225285_at
phosphoprotein associated with glycosphingolipid-enriched microdomains	Consensus includes gb:AK000680.1 /DEF=Homo sapiens cDNA FLJ20673 fis, clone KAIA4464. /FEA=mRNA /DB_XREF=gi:7020924 /UG=Hs.266175 phosphoprotein associated with GEMs /FL=gb:AF240634.1 gb:NM_018440.1	225626_at
hypothetical protein FLJ20209	Consensus includes gb:BF111925 /FEA=EST /DB_XREF=gi:10941704 /DB_XREF=est:7l38g05.x1 /CLONE=IMAGE:3523784 /UG=Hs.3685 hypothetical protein FLJ20209	226171_at
Homo sapiens mRNA for KIAA1190 protein, partial cds	Consensus includes gb:AA532640 /FEA=EST /DB_XREF=gi:2276894 /DB_XREF=est:nj17c04.s1 /CLONE=IMAGE:986598 /UG=Hs.206259 Homo sapiens mRNA for KIAA1190 protein, partial cds	226484_at
KIAA1543 protein	Consensus includes gb:AB040976.1 /DEF=Homo sapiens mRNA for KIAA1543 protein, partial cds. /FEA=mRNA /GEN=KIAA1543 /PROD=KIAA1543 protein /DB_XREF=gi:7959352 /UG=Hs.17686 KIAA1543 protein	226494_at
hypothetical protein FLJ23563	Consensus includes gb:AW138767 /FEA=EST /DB_XREF=gi:6143085 /DB_XREF=est:UI-H-BI1-aep-a-12-0-UI.s1 /CLONE=IMAGE:2719799 /UG=Hs.274256 hypothetical protein FLJ23563	227180_at
ESTs	Consensus includes gb:AW264333 /FEA=EST /DB_XREF=gi:6641075 /DB_XREF=est:xq98e01.x1	227320_at

	/CLONE=IMAGE:2758680 /UG=Hs.21835 ESTs	
ESTs	Consensus includes gb:BF589359 /FEA=EST /DB_XREF=gi:11681683 /DB_XREF=est:nab25d01.x1 /CLONE=IMAGE:3266737 /UG=Hs.13256 ESTs	227354_at
Homo sapiens, Similar to RIKEN cDNA 1810037C20 gene, clone MGC:21481 IMAGE:3852062, mRNA, complete cds	Consensus includes gb:AW001287 /FEA=EST /DB_XREF=gi:5848203 /DB_XREF=est:wu27e06.x1 /CLONE=IMAGE:2521282 /UG=Hs.61265 ESTs, Weakly similar to G786_HUMAN PROTEIN GS3786 H.sapiens	227676_at
Homo sapiens cDNA: FLJ22063 fis, clone HEP10326	Consensus includes gb:T86159 /FEA=EST /DB_XREF=gi:714511 /DB_XREF=est:yd84h07.s1 /CLONE=IMAGE:114973 /UG=Hs.10450 Homo sapiens cDNA: FLJ22063 fis, clone HEP10326	227724_at
ESTs	Consensus includes gb:AI700341 /FEA=EST /DB_XREF=gi:4988241 /DB_XREF=est:wd06e10.x1 /CLONE=IMAGE:2327370 /UG=Hs.110406 ESTs	228653_at
ESTs	Consensus includes gb:BG494007 /FEA=EST /DB_XREF=gi:13455521 /DB_XREF=est:602542289F1 /CLONE=IMAGE:4673182 /UG=Hs.203213 ESTs	228716_at
ESTs	Consensus includes gb:AI559300 /FEA=EST /DB_XREF=gi:4509505 /DB_XREF=est:tq43d03.x1 /CLONE=IMAGE:2211557 /UG=Hs.294140 ESTs	229331_at
hypothetical protein	Consensus includes gb:AI830823 /FEA=EST /DB_XREF=gi:5451416 /DB_XREF=est:wj52b06.x1 /CLONE=IMAGE:2406419 /UG=Hs.95549 hypothetical protein	229439_s_at
ESTs	Consensus includes gb:BF431989 /FEA=EST /DB_XREF=gi:11444103 /DB_XREF=est:nab84a05.x1 /CLONE=IMAGE:3274280 /UG=Hs.203213 ESTs	229657_at
ESTs	Consensus includes gb:BF589413 /FEA=EST	229893_at

	/DB_XREF=gi:11681737 /DB_XREF=est:nab26b11.x1 /CLONE=IMAGE:3267020 /UG=Hs.55501 ESTs	
brain-specific protein p25 alpha	Consensus includes gb:BG055052 /FEA=EST /DB_XREF=gi:12512386 /DB_XREF=est:nac94g06.x1 /CLONE=IMAGE:3441995 /UG=Hs.29353 brain-specific protein p25 alpha	230104_s_at
ESTs, Weakly similar to MMHUE4 erythrocyte membrane protein 4.1, parent splice form [H.sapiens]	Consensus includes gb:BF110588 /FEA=EST /DB_XREF=gi:10940278 /DB_XREF=est:7n39e12.x1 /CLONE=IMAGE:3567071 /UG=Hs.150478 ESTs, Weakly similar to KIAA0987 protein H.sapiens	230645_at
ESTs	Consensus includes gb:BF592062 /FEA=EST /DB_XREF=gi:11684386 /DB_XREF=est:7n98h06.x1 /CLONE=IMAGE:3572962 /UG=Hs.233890 ESTs	230760_at
hepatocyte nuclear factor 4, alpha	Consensus includes gb:AI032108 /FEA=EST /DB_XREF=gi:3250320 /DB_XREF=est:ow92d11.x1 /CLONE=IMAGE:1654293 /UG=Hs.54424 hepatocyte nuclear factor 4, alpha	230914_at
ESTs	Consensus includes gb:AW203959 /FEA=EST /DB_XREF=gi:6503431 /DB_XREF=est:UI-H-BI1-aeu-b-12-0-UI.s1 /CLONE=IMAGE:2720590 /UG=Hs.149532 ESTs	230944_at
ESTs	Consensus includes gb:AI139990 /FEA=EST /DB_XREF=gi:3647447 /DB_XREF=est:qa47d03.x1 /CLONE=IMAGE:1689893 /UG=Hs.134586 ESTs	231022_at
ESTs	Consensus includes gb:AI806131 /FEA=EST /DB_XREF=gi:5392697 /DB_XREF=est:wf06c06.x1 /CLONE=IMAGE:2349802 /UG=Hs.99376 ESTs	231148_at
hypothetical protein FLJ23045	Consensus includes gb:AB046810.1 /DEF=Homo sapiens mRNA for KIAA1590 protein, partial cds. /FEA=mRNA /GEN=KIAA1590 /PROD=KIAA1590 protein /DB_XREF=gi:10047254 /UG=Hs.101774 hypothetical protein FLJ23045	232083_at

Homo sapiens PAC clone RP5-855D21	Consensus includes gb:AC004908 /DEF=Homo sapiens PAC clone RP5-855D21 /FEA=CDS_3 /DB_XREF=gi:4156179 /UG=Hs.249181 Homo sapiens PAC clone RP5-855D21	232641_at
putative microtubule-binding protein	Consensus includes gb:AJ251708.1 /DEF=Homo sapiens partial mRNA for putative microtubule-binding protein. /FEA=mRNA /PROD=putative microtubule-binding protein /DB_XREF=gi:6491740 /UG=Hs.326544 putative microtubule-binding protein	234669_x_at
ESTs	Consensus includes gb:AI741469 /FEA=EST /DB_XREF=gi:5109757 /DB_XREF=est:wg11b01.x1 /CLONE=IMAGE:2364745 /UG=Hs.57787 ESTs	234970_at
ESTs	Consensus includes gb:AI417897 /FEA=EST /DB_XREF=gi:4261401 /DB_XREF=est:tg55b06.x1 /CLONE=IMAGE:2112659 /UG=Hs.235860 ESTs	235444_at
ESTs	Consensus includes gb:AI493909 /FEA=EST /DB_XREF=gi:4394912 /DB_XREF=est:qz94e02.x1 /CLONE=IMAGE:2042234 /UG=Hs.6131 ESTs	235562_at
ESTs	Consensus includes gb:AV741130 /FEA=EST /DB_XREF=gi:10858711 /DB_XREF=est:AV741130 /CLONE=CBCATB06 /UG=Hs.173704 ESTs, Moderately similar to ALU8_HUMAN ALU SUBFAMILY SX SEQUENCE CONTAMINATION WARNING ENTRY H.sapiens	235651_at
ESTs	Consensus includes gb:AW339510 /FEA=EST /DB_XREF=gi:6836136 /DB_XREF=est:xz91h08.x1 /CLONE=IMAGE:2871615 /UG=Hs.42722 ESTs	235866_at
ESTs	Consensus includes gb:AI076192 /FEA=EST /DB_XREF=gi:3405370 /DB_XREF=est:oz01g07.x1 /CLONE=IMAGE:1674108 /UG=Hs.131933 ESTs	236422_at
ESTs	Consensus includes gb:AL044570 /FEA=EST /DB_XREF=gi:5432785 /DB_XREF=est:DKFZp434L082_s1 /CLONE=DKFZp434L082 /UG=Hs.147975 ESTs	236548_at

ESTs	Consensus includes gb:AI733801 /FEA=EST /DB_XREF=gi:5054914 /DB_XREF=est:qk39c04.x5 /CLONE=IMAGE:1871334 /UG=Hs.146186 ESTs	237923_at
Homo sapiens, clone MGC:16402 IMAGE:3940360, mRNA, complete cds	Consensus includes gb:T69015 /FEA=EST /DB_XREF=gi:680163 /DB_XREF=est:yc31f04.s1 /CLONE=IMAGE:82303 /UG=Hs.192728 ESTs	238422_at
ESTs	Consensus includes gb:AA502384 /FEA=EST /DB_XREF=gi:2237351 /DB_XREF=est:ne27f11.s1 /CLONE=IMAGE:898605 /UG=Hs.151529 ESTs	238956_at
ESTs	Consensus includes gb:AI739241 /FEA=EST /DB_XREF=gi:5101222 /DB_XREF=est:wi14h02.x1 /CLONE=IMAGE:2390259 /UG=Hs.171480 ESTs	238984_at
ESTs	Consensus includes gb:AA088446 /FEA=EST /DB_XREF=gi:1633958 /DB_XREF=est:zl89f04.s1 /CLONE=IMAGE:511807 /UG=Hs.170298 ESTs	239065_at
ESTs	Consensus includes gb:AI493046 /FEA=EST /DB_XREF=gi:4394049 /DB_XREF=est:qz49b04.x1 /CLONE=IMAGE:2030191 /UG=Hs.146133 ESTs	239148_at
ESTs	Consensus includes gb:AI243098 /FEA=EST /DB_XREF=gi:3838495 /DB_XREF=est:qh26e03.x1 /CLONE=IMAGE:1845820 /UG=Hs.178398 ESTs	239966_at
ESTs, Weakly similar to A49175 Motch B protein - mouse [M.musculus]	Consensus includes gb:AI633523 /FEA=EST /DB_XREF=gi:4684853 /DB_XREF=est:th68b11.x1 /CLONE=IMAGE:2123805 /UG=Hs.44705 ESTs	240106_at
betacellulin	Consensus includes gb:AI620677 /FEA=EST /DB_XREF=gi:4629803 /DB_XREF=est:tu85e09.x1 /CLONE=IMAGE:2257864 /UG=Hs.154191 ESTs	241412_at
ESTs	Consensus includes gb:BF696216 /FEA=EST /DB_XREF=gi:11981624	242626_at

	/DB_XREF=est:602124536F1 /CLONE=IMAGE:4281632 /UG=Hs.188724 ESTs	
ESTs	Consensus includes gb:N57929 /FEA=EST /DB_XREF=gi:1201819 /DB_XREF=est:yv61e06.s1 /CLONE=IMAGE:247234 /UG=Hs.48100 ESTs	242978_x_at
ESTs, Weakly similar to ALU1_HUMAN ALU SUBFAMILY J SEQUENCE CONTAMINATION WARNING ENTRY [H.sapiens]	Consensus includes gb:AI457984 /FEA=EST /DB_XREF=gi:4312002 /DB_XREF=est:tj66a04.x1 /CLONE=IMAGE:2146446 /UG=Hs.165900 ESTs, Weakly similar to ALUC_HUMAN !!!! ALU CLASS C WARNING ENTRY !!! H.sapiens	243729_at
ESTs	Consensus includes gb:AA581439 /FEA=EST /DB_XREF=gi:2359211 /DB_XREF=est:nh13c10.s1 /CLONE=IMAGE:952242 /UG=Hs.152328 ESTs	244650_at

The two biomarker probe sets A and B were then combined, a total of 161 different probe sets, and the redundant polynucleotides were removed, representing 125 unique polynucleotides which are provided below in Table 4. The Table 4 polynucleotides are biomarkers of the invention.

TABLE 4 - Biomarkers

Unigene Title And SEQ ID NO:	Affymetrix Description	Affymetrix probe set
3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2 (mitochondrial) SEQ ID NOS: 1 (DNA) and 126 (amino acid)	gb:NM_005518.1 /DEF=Homo sapiens 3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2 (mitochondrial) (HMGCS2), mRNA. /FEA=mRNA /GEN=HMGCS2 /PROD=3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2(mitochondrial) /DB_XREF=gi:5031750 /UG=Hs.59889 3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2 (mitochondrial) /FL=gb:NM_005518.1	204607_at
ATPase, Class V, type 10B	Consensus includes gb:AW006935 /FEA=EST /DB_XREF=gi:5855713 /DB_XREF=est:wt08b11.x1	214070_s_at

SEQ ID NO: 2 (DNA)	/CLONE=IMAGE:2506845 /UG=Hs.109358 ATPase, Class V, type 10B	
bone morphogenetic protein 2 SEQ ID NOS: 3 (DNA) and 127 (amino acid)	gb:NM_001200.1 /DEF=Homo sapiens bone morphogenetic protein 2 (BMP2), mRNA. /FEA=mRNA /GEN=BMP2 /PROD=bone morphogenetic protein 2 precursor /DB_XREF=gi:4557368 /UG=Hs.73853 bone morphogenetic protein 2 /FL=gb:NM_001200.1	205290_s_at
brain-specific protein p25 alpha SEQ ID NOS: 4 (DNA) and 128 (amino acid)	gb:NM_007030.1 /DEF=Homo sapiens brain-specific protein p25 alpha (p25), mRNA. /FEA=mRNA /GEN=p25 /PROD=brain-specific protein p25 alpha /DB_XREF=gi:5902017 /UG=Hs.29353 brain-specific protein p25 alpha /FL=gb:AB017016.1 gb:NM_007030.1	206179_s_at
branched chain aminotransferase 1, cytosolic SEQ ID NOS: 5 (DNA) and 129 (amino acid)	Consensus includes gb:NM_005504.1 /DEF=Homo sapiens branched chain aminotransferase 1, cytosolic (BCAT1), mRNA. /FEA=CDS /GEN=BCAT1 /PROD=branched chain aminotransferase 1, cytosolic /DB_XREF=gi:5031606 /UG=Hs.157205 branched chain aminotransferase 1, cytosolic /FL=gb:U21551.1 gb:NM_005504.1	214452_at
BTG family, member 2 SEQ ID NOS: 6 (DNA) and 130 (amino acid)	gb:NM_006763.1 /DEF=Homo sapiens BTG family, member 2 (BTG2), mRNA. /FEA=mRNA /GEN=BTG2 /PROD=BTG family, member 2 /DB_XREF=gi:5802987 /UG=Hs.75462 BTG family, member 2 /FL=gb:U72649.1 gb:NM_006763.1	201236_s_at
Carcinoembryonic antigen-related cell adhesion molecule 6 (non-specific cross reacting antigen) SEQ ID NOS: 7 (DNA) and 131 (amino acid)	gb:BC005008.1 /DEF=Homo sapiens, carcinoembryonic antigen-related cell adhesion molecule 6 (non-specific cross reacting antigen), clone MGC:10467, mRNA, complete cds. /FEA=mRNA /PROD=carcinoembryonic antigen-related cell adhesionmolecule 6 (non-specific cross reacting antigen) /DB_XREF=gi:13477106 /UG=Hs.73848 carcinoembryonic antigen-related cell adhesion molecule 6 (non-specific cross reacting antigen) /FL=gb:BC005008.1 gb:M18216.1 gb:M29541.1 gb:NM_002483.1	203757_s_at
caspase 10, apoptosis-related cysteine protease SEQ ID NOS: 8	gb:NM_001230.1 /DEF=Homo sapiens caspase 10, apoptosis-related cysteine protease (CASP10), mRNA. /FEA=mRNA /GEN=CASP10 /PROD=caspase 10, apoptosis-related cysteine protease	205467_at

(DNA) and 132 (amino acid)	/DB_XREF=gi:4502568 /UG=Hs.5353 caspase 10, apoptosis-related cysteine protease /FL=gb:U60519.1 gb:NM_001230.1	
CUG triplet repeat, RNA-binding protein 2 SEQ ID NOS: 9 (DNA) and 133 (amino acid)	gb:NM_006561.1 /DEF=Homo sapiens CUG triplet repeat, RNA-binding protein 2 (CUGBP2), mRNA. /FEA=mRNA /GEN=CUGBP2 /PROD=CUG triplet repeat, RNA-binding protein 2 /DB_XREF=gi:5729815 /UG=Hs.211610 CUG triplet repeat, RNA-binding protein 2 /FL=gb:U69546.1 gb:AF036956.1 gb:AF090694.1 gb:NM_006561.1	202158_s_at
cystatin S SEQ ID NOS: 10 (DNA) and 134 (amino acid)	gb:NM_001899.1 /DEF=Homo sapiens cystatin S (CST4), mRNA. /FEA=mRNA /GEN=CST4 /PROD=cystatin S /DB_XREF=gi:4503108 /UG=Hs.56319 cystatin S /FL=gb:NM_001899.1	206994_at
cystic fibrosis transmembrane conductance regulator, ATP-binding cassette (sub-family C, member 7) SEQ ID NOS: 11 (DNA) and 135 (amino acid)	gb:NM_000492.2 /DEF=Homo sapiens cystic fibrosis transmembrane conductance regulator, ATP-binding cassette (sub-family C, member 7) (CFTR), mRNA. /FEA=mRNA /GEN=CFTR /PROD=cystic fibrosis transmembrane conductanceregulator, ATP-binding cassette (sub-family C, member 7) /DB_XREF=gi:6995995 /UG=Hs.663 cystic fibrosis transmembrane conductance regulator, ATP-binding cassette (sub-family C, member 7) /FL=gb:NM_000492.2	205043_at
cytochrome P450, subfamily IIJ (arachidonic acid epoxygenase) polypeptide 2 SEQ ID NOS: 12 (DNA) and 136 (amino acid)	gb:NM_000775.1 /DEF=Homo sapiens cytochrome P450, subfamily IIJ (arachidonic acid epoxygenase) polypeptide 2 (CYP2J2), mRNA. /FEA=mRNA /GEN=CYP2J2 /PROD=cytochrome P450, subfamily IIJ (arachidonic acidepoxygenase) polypeptide 2 /DB_XREF=gi:4503226 /UG=Hs.152096 cytochrome P450, subfamily IIJ (arachidonic acid epoxygenase) polypeptide 2 /FL=gb:U37143.1 gb:NM_000775.1	205073_at
dipeptidylpeptidase IV (CD26, adenosine deaminase complexing protein 2) SEQ ID NOS 13 (DNA) and 137 (amino acid)	gb:M80536.1 /DEF=H.sapiens dipeptidyl peptidase IV (DPP4) mRNA, complete cds. /FEA=mRNA /GEN=DPP4 /PROD=dipeptidyl peptidase IV /DB_XREF=gi:181569 /UG=Hs.44926 dipeptidylpeptidase IV (CD26, adenosine deaminase complexing protein 2) /FL=gb:M80536.1 gb:NM_001935.1	203716_s_at
DKFZP434C091 protein	Consensus includes gb:AL080170.1 /DEF=Homo sapiens mRNA; cDNA	215047_at

SEQ ID NO: 14 (DNA)	DKFZp434C091 (from clone DKFZp434C091); partial cds. /FEA=mRNA /GEN=DKFZp434C091 /PROD=hypothetical protein /DB_XREF=gi:5262639 /UG=Hs.51692 DKFZP434C091 protein	
dopa decarboxylase (aromatic L-amino acid decarboxylase) SEQ ID NO: 15 (DNA)	Consensus includes gb:AW772056 /FEA=EST /DB_XREF=gi:7704118 /DB_XREF=est:hn64g06.x1 /CLONE=IMAGE:3032698 /UG=Hs.150403 dopa decarboxylase (aromatic L-amino acid decarboxylase)	214347_s_at
EphA1 SEQ ID NOS: 16 (DNA) and 138 (amino acid)	gb:NM_005232.1 /DEF=Homo sapiens EphA1 (EPHA1), mRNA. /FEA=mRNA /GEN=EPHA1 /PROD=EphA1 /DB_XREF=gi:4885208 /UG=Hs.89839 EphA1 /FL=gb:M18391.1 gb:NM_005232.1	205977_s_at
ESTs, Moderately similar to AF078844 1 hqp0376 protein [H.sapiens] SEQ ID NO: 17 (DNA)	Consensus includes gb:R06655 /FEA=EST /DB_XREF=gi:757275 /DB_XREF=est:yf10e02.r1 /CLONE=IMAGE:126458 /UG=Hs.188518 ESTs, Moderately similar to AF078844 1 hqp0376 protein H.sapiens	217546_at
ESTs, Weakly similar to I38022 hypothetical protein [H.sapiens] SEQ ID NO: 18 (DNA)	Consensus includes gb:AW675655 /FEA=EST /DB_XREF=gi:7540890 /DB_XREF=est:ba52e01.x1 /CLONE=IMAGE:2900184 /UG=Hs.314158 ESTs	222354_at
fibroblast growth factor receptor 2 (bacteria-expressed kinase, keratinocyte growth factor receptor, craniofacial dysostosis 1, Crouzon syndrome, Pfeiffer syndrome, Jackson-Weiss syndrome) SEQ ID NOS: 19 (DNA) and 139 (amino acid)	gb:NM_022969.1 /DEF=Homo sapiens fibroblast growth factor receptor 2 (bacteria-expressed kinase, keratinocyte growth factor receptor, craniofacial dysostosis 1, Crouzon syndrome, Pfeiffer syndrome, Jackson-Weiss syndrome) (FGFR2), transcript variant 2, mRNA. /FEA=mRNA /GEN=FGFR2 /PROD=fibroblast growth factor receptor 2, isoform 2precursor /DB_XREF=gi:13186252 /UG=Hs.278581 fibroblast growth factor receptor 2 (bacteria-expressed kinase, keratinocyte growth factor receptor, craniofacial dysostosis 1, Crouzon syndrome, Pfeiffer syndrome, Jackson-Weiss syndrome) /FL=gb:NM_022969.1 gb:M97193.1 gb:M80634.1	203638_s_at
FXYD domain-containing ion	gb:BC005238.1 /DEF=Homo sapiens, FXYD domain-containing ion transport regulator 3,	202489_s_at

transport regulator 3 SEQ ID NOS: 20 (DNA) and 140 (amino acid)	clone MGC:12265, mRNA, complete cds. /FEA=mRNA /PROD=FXYD domain-containing ion transport regulator3 /DB_XREF=gi:13528881 /UG=Hs.301350 FXYD domain-containing ion transport regulator 3 /FL=gb:NM_005971.2 gb:BC005238.1	
G protein-coupled receptor 49 SEQ ID NOS: 21 (DNA) and 141 (amino acid)	gb:AF062006.1 /DEF=Homo sapiens orphan G protein-coupled receptor HG38 mRNA, complete cds. /FEA=mRNA /PROD=orphan G protein-coupled receptor HG38 /DB_XREF=gi:3366801 /UG=Hs.285529 G protein-coupled receptor 49 /FL=gb:AF062006.1 gb:AF061444.1 gb:NM_003667.1	210393_at
hairless (mouse) homolog SEQ ID NOS: 22 (DNA) and 142 (amino acid)	gb:NM_018411.1 /DEF=Homo sapiens hairless protein (putative single zinc finger transcription factor protein, responsible for autosomal recessive universal congenital alopecia, HR gene) (HSA277165), mRNA. /FEA=mRNA /GEN=HSA277165 /PROD=hairless protein /DB_XREF=gi:11036651 /UG=Hs.272367 hairless protein (putative single zinc finger transcription factor protein, responsible for autosomal recessive universal congenital alopecia, HR gene) /FL=gb:NM_018411.1	220163_s_at
hemoglobin, alpha 1 SEQ ID NOS: 23 (DNA) and 143 (amino acid)	gb:BC005931.1 /DEF=Homo sapiens, hemoglobin, alpha 2, clone MGC:14541, mRNA, complete cds. /FEA=mRNA /PROD=hemoglobin, alpha 2 /DB_XREF=gi:13543547 /FL=gb:BC005931.1	211745_x_at
hemoglobin, alpha 2 SEQ ID NO: 24 (DNA)	Consensus includes gb:T50399 /FEA=EST /DB_XREF=gi:652259 /DB_XREF=est:yb30b11.s1 /CLONE=IMAGE:72669 /UG=Hs.251577 hemoglobin, alpha 1	214414_x_at
heparanase SEQ ID NOS: 25 (DNA) and 144 (amino acid)	gb:NM_006665.1 /DEF=Homo sapiens heparanase (HPSE), mRNA. /FEA=mRNA /GEN=HPSE /PROD=heparanase /DB_XREF=gi:5729872 /UG=Hs.44227 heparanase /FL=gb:AF165154.1 gb:AF152376.1 gb:NM_006665.1 gb:AF084467.1 gb:AF155510.1	219403_s_at
Hermansky-Pudlak syndrome	Consensus includes gb:AL022313 /DEF=Human DNA sequence from clone RP5-1119A7 on chromosome 22q12.2-12.3	217354_s_at

SEQ ID NOS: 26 (DNA) and 145 (amino acid)	Contains the TXN2 gene for mitochondrial thioredoxin, a novel gene, the EIF3S7 gene for eukaryotic translation initiation factor 3 subunit 7 (zeta, 6667kD) (EIF3-P66), the gene f... /FEA=CDS_3 /DB_XREF=gi:4200326 /UG=Hs.272270 Human DNA sequence from clone RP5-1119A7 on chromosome 22q12.2-12.3 Contains the TXN2 gene for mitochondrial thioredoxin, a novel gene, the EIF3S7 gene for eukaryotic translation initiation factor 3 subunit 7 (zeta, 6667kD) (EIF3-P66), the gene for a nov	
HERV-H LTR-associating 2 SEQ ID NOS: 27 (DNA) and 146 (amino acid)	gb:NM_007072.1 /DEF=Homo sapiens HERV-H LTR-associating 2 (HHLA2), mRNA. /FEA=mRNA /GEN=HHLA2 /PROD=HERV-H LTR-associating 2 /DB_XREF=gi:5901963 /UG=Hs.252351 HERV-H LTR-associating 2 /FL=gb:AF126162.1 gb:NM_007072.1	220812_s_at
Homo sapiens clone 24707 mRNA sequence SEQ ID NO: 28 (DNA)	Consensus includes gb:AW593996 /FEA=EST /DB_XREF=gi:7281254 /DB_XREF=est:hg41g06.x1 /CLONE=IMAGE:2948218 /UG=Hs.124969 Homo sapiens clone 24707 mRNA sequence	213256_at
Homo sapiens mRNA; cDNA DKFZp564D042 (from clone DKFZp564D042) SEQ ID NO: 29 (DNA)	Consensus includes gb:AL049983.1 /DEF=Homo sapiens mRNA; cDNA DKFZp564D042 (from clone DKFZp564D042). /FEA=mRNA /DB_XREF=gi:4884234 /UG=Hs.240136 Homo sapiens mRNA; cDNA DKFZp564D042 (from clone DKFZp564D042)	217288_at
hypothetical protein FLJ20048 SEQ ID NOS: 30 (DNA) and 147 (amino acid)	gb:NM_017640.1 /DEF=Homo sapiens hypothetical protein FLJ20048 (FLJ20048), mRNA. /FEA=mRNA /GEN=FLJ20048 /PROD=hypothetical protein FLJ20048 /DB_XREF=gi:8923056 /UG=Hs.116470 hypothetical protein FLJ20048 /FL=gb:NM_017640.1	219573_at
hypothetical protein FLJ20075 SEQ ID NOS: 31 (DNA) and 148 (amino acid)	gb:NM_017655.1 /DEF=Homo sapiens hypothetical protein FLJ20075 (FLJ20075), mRNA. /FEA=mRNA /GEN=FLJ20075 /PROD=hypothetical protein FLJ20075 /DB_XREF=gi:8923083 /UG=Hs.205058 hypothetical protein FLJ20075 /FL=gb:NM_017655.1	219970_at

interferon consensus sequence binding protein 1 SEQ ID NO: 32 (DNA)	Consensus includes gb:AI073984 /FEA=EST /DB_XREF=gi:3400628 /DB_XREF=est:oy66c05.x1 /CLONE=IMAGE:1670792 /UG=Hs.14453 interferon consensus sequence binding protein 1 /FL=gb:M91196.1 gb:NM_002163.1	204057_at
KIAA0690 protein SEQ ID NO: 33 (DNA)	Consensus includes gb:AK000238.1 /DEF=Homo sapiens cDNA FLJ20231 fis, clone COLF5511, highly similar to AB014590 Homo sapiens mRNA for KIAA0690 protein. /FEA=mRNA /DB_XREF=gi:7020188 /UG=Hs.60103 KIAA0690 protein	216360_x_at
matrilin 3 SEQ ID NOS: 34 (DNA) and 149 (amino acid)	gb:NM_002381.2 /DEF=Homo sapiens matrilin 3 (MATN3) precursor, mRNA. /FEA=mRNA /GEN=MATN3 /PROD=matrilin 3 precursor /DB_XREF=gi:13518040 /UG=Hs.278461 matrilin 3 /FL=gb:NM_002381.2	206091_at
metastasis-associated 1-like 1 SEQ ID NOS: 35 (DNA) and 150 (amino acid)	gb:NM_004739.1 /DEF=Homo sapiens metastasis-associated 1-like 1 (MTA1L1), mRNA. /FEA=mRNA /GEN=MTA1L1 /PROD=metastasis-associated 1-like 1 /DB_XREF=gi:4758739 /UG=Hs.173043 metastasis-associated 1-like 1 /FL=gb:AB016591.1 gb:NM_004739.1 gb:AF295807.1	203444_s_at
mucin 2, intestinal/tracheal SEQ ID NOS: 36 (DNA) and 151 (amino acid)	gb:NM_002457.1 /DEF=Homo sapiens mucin 2, intestinaltracheal (MUC2), mRNA. /FEA=mRNA /GEN=MUC2 /PROD=mucin 2, intestinaltracheal /DB_XREF=gi:4505284 /UG=Hs.315 mucin 2, intestinaltracheal /FL=gb:NM_002457.1 gb:L21998.1	204673_at
mucin 3B SEQ ID NOS: 37 (DNA) and 152 (amino acid)	Consensus includes gb:AB038783.1 /DEF=Homo sapiens MUC3B mRNA for intestinal mucin, partial cds. /FEA=mRNA /GEN=MUC3B /PROD=intestinal mucin /DB_XREF=gi:9929917 /UG=Hs.129782 mucin 3A, intestinal	214898_x_at
myosin, heavy polypeptide 13, skeletal muscle SEQ ID NOS: 38 (DNA) and 153 (amino acid)	gb:NM_003802.1 /DEF=Homo sapiens myosin, heavy polypeptide 13, skeletal muscle (MYH13), mRNA. /FEA=mRNA /GEN=MYH13 /PROD=myosin, heavy polypeptide 13, skeletal muscle /DB_XREF=gi:11321578 /UG=Hs.278488 myosin, heavy polypeptide 13, skeletal muscle /FL=gb:NM_003802.1 gb:AF111782.2	208208_at

myosin, light polypeptide 5, regulatory SEQ ID NOS: 39 (DNA) and 154 (amino acid)	gb:NM_002477.1 /DEF=Homo sapiens myosin, light polypeptide 5, regulatory (MYL5), mRNA. /FEA=mRNA /GEN=MYL5 /PROD=myosin, light polypeptide 5, regulatory /DB_XREF=gi:4505304 /UG=Hs.170482 myosin, light polypeptide 5, regulatory /FL=gb:L03785.1 gb:NM_002477.1	205145_s_at
nuclear receptor subfamily 3, group C, member 2 SEQ ID NOS: 40 (DNA) and 155 (amino acid)	gb:NM_000901.1 /DEF=Homo sapiens nuclear receptor subfamily 3, group C, member 2 (NR3C2), mRNA. /FEA=mRNA /GEN=NR3C2 /PROD=nuclear receptor subfamily 3, group C, member 2 /DB_XREF=gi:4505198 /UG=Hs.1790 nuclear receptor subfamily 3, group C, member 2 /FL=gb:M16801.1 gb:NM_000901.1	205259_at
nuclear receptor subfamily 5, group A, member 2 SEQ ID NOS: 41 (DNA) and 156 (amino acid)	Consensus includes gb:AF228413.1 /DEF=Homo sapiens hepatocyte transcription factor mRNA, 3UTR. /FEA=mRNA /DB_XREF=gi:7677372 /UG=Hs.183123 nuclear receptor subfamily 5, group A, member 2 /FL=gb:U93553.1 gb:AB019246.1 gb:AF124247.1	210174_at
pancreas-enriched phospholipase C SEQ ID NOS: 42 (DNA) and 157 (amino acid)	gb:NM_016341.1 /DEF=Homo sapiens pancreas-enriched phospholipase C (LOC51196), mRNA. /FEA=mRNA /GEN=LOC51196 /PROD=pancreas-enriched phospholipase C /DB_XREF=gi:7705940 /UG=Hs.6733 pancreas-enriched phospholipase C /FL=gb:AF190642.2 gb:AF117948.1 gb:NM_016341.1	205112_at
peroxisomal trans 2-enoyl CoA reductase; putative short chain alcohol dehydrogenase SEQ ID NOS: 43 (DNA) and 158 (amino acid)	gb:NM_018441.1 /DEF=Homo sapiens peroxisomal trans 2-enoyl CoA reductase; putative short chain alcohol dehydrogenase (HSA250303), mRNA. /FEA=mRNA /GEN=HSA250303 /PROD=peroxisomal trans 2-enoyl CoA reductase;putative short chain alcohol dehydrogenase /DB_XREF=gi:8923751 /UG=Hs.281680 peroxisomal trans 2-enoyl CoA reductase; putative short chain alcohol dehydrogenase /FL=gb:NM_018441.1	221142_s_at
phosducin SEQ ID NOS: 44 (DNA) and 159 (amino acid)	gb:M33478.1 /DEF=Human 33-kDa phototransducing protein mRNA, complete cds. /FEA=mRNA /DB_XREF=gi:177186 /UG=Hs.550 phosducin /FL=gb:NM_022577.1 gb:M33478.1	211496_s_at

	gb:AF076465.1	
phosphatase and tensin homolog (mutated in multiple advanced cancers 1) SEQ ID NOS: 45 (DNA) and 160 (amino acid)	gb:NM_000314.1 /DEF=Homo sapiens phosphatase and tensin homolog (mutated in multiple advanced cancers 1) (PTEN), mRNA. /FEA=mRNA /GEN=PTEN /PROD=phosphatase and tensin homolog (mutated inmultiple advanced cancers 1) /DB_XREF=gi:4506248 /UG=Hs.10712 phosphatase and tensin homolog (mutated in multiple advanced cancers 1) /FL=gb:U92436.1 gb:U93051.1 gb:U96180.1 gb:NM_000314.1	204054_at
potassium channel, subfamily K, member 1 (TWIK-1) SEQ ID NOS: 46 (DNA) and 161 (amino acid)	gb:U90065.1 /DEF=Human potassium channel KCNO1 mRNA, complete cds. /FEA=mRNA /PROD=potassium channel KCNO1 /DB_XREF=gi:1916294 /UG=Hs.79351 potassium channel, subfamily K, member 1 (TWIK-1) /FL=gb:U33632.1 gb:U90065.1 gb:U76996.1 gb:NM_002245.1	204678_s_at
prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase) SEQ ID NOS: 47 (DNA) and 162 (amino acid)	gb:NM_000963.1 /DEF=Homo sapiens prostaglandin-endoperoxide synthase 2 (prostaglandin GH synthase and cyclooxygenase) (PTGS2), mRNA. /FEA=mRNA /GEN=PTGS2 /PROD=prostaglandin-endoperoxide synthase 2(prostaglandin GH synthase and cyclooxygenase) /DB_XREF=gi:4506264 /UG=Hs.196384 prostaglandin-endoperoxide synthase 2 (prostaglandin GH synthase and cyclooxygenase) /FL=gb:M90100.1 gb:L15326.1 gb:NM_000963.1	204748_at
protease inhibitor 3, skin-derived (SKALP) SEQ ID NOS: 48 (DNA) and 163 (amino acid)	gb:NM_002638.1 /DEF=Homo sapiens protease inhibitor 3, skin-derived (SKALP) (PI3), mRNA. /FEA=mRNA /GEN=PI3 /PROD=protease inhibitor 3, skin-derived (SKALP) /DB_XREF=gi:4505786 /UG=Hs.112341 protease inhibitor 3, skin-derived (SKALP) /FL=gb:NM_002638.1	203691_at
PTPRF interacting protein, binding protein 2 (liprin beta 2) SEQ ID NO: 49 (DNA)	Consensus includes gb:AI692180 /FEA=EST /DB_XREF=gi:4969520 /DB_XREF=est:wd37f06.x1 /CLONE=IMAGE:2330339 /UG=Hs.12953 PTPRF interacting protein, binding protein 2 (liprin beta 2)	212841_s_at
retinoic acid receptor responder (tazarotene induced) 1	Consensus includes gb:AI669229 /FEA=EST /DB_XREF=gi:4834003 /DB_XREF=est:wc13e06.x1	221872_at

SEQ ID NO: 50 (DNA)	/CLONE=IMAGE:2315074 /UG=Hs.82547 retinoic acid receptor responder (tazarotene induced) 1	
Rho GTPase activating protein 8 SEQ ID NOS: 51 (DNA) and 164 (amino acid)	gb:NM_015366.1 /DEF=Homo sapiens Rho GTPase activating protein 8 (ARHGAP8), mRNA. /FEA=mRNA /GEN=ARHGAP8 /PROD=Rho GTPase activating protein 8 /DB_XREF=gi:7656903 /UG=Hs.102336 Rho GTPase activating protein 8 /FL=gb:NM_015366.1	205980_s_at
ribonuclease, RNase A family, 1 (pancreatic) SEQ ID NOS: 52 (DNA) and 165 (amino acid)	gb:NM_002933.1 /DEF=Homo sapiens ribonuclease, RNase A family, 1 (pancreatic) (RNASE1), mRNA. /FEA=mRNA /GEN=RNASE1 /PROD=ribonuclease, RNase A family, 1 (pancreatic) /DB_XREF=gi:4506546 /UG=Hs.78224 ribonuclease, RNase A family, 1 (pancreatic) /FL=gb:BC005324.1 gb:NM_002933.1 gb:D26129.1	201785_at
serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 5 SEQ ID NOS: 53 (DNA) and 166 (amino acid)	gb:NM_002639.1 /DEF=Homo sapiens serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 5 (SERPINB5), mRNA. /FEA=mRNA /GEN=SERPINB5 /PROD=serine (or cysteine) proteinase inhibitor, cladeB (ovalbumin), member 5 /DB_XREF=gi:4505788 /UG=Hs.55279 serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 5 /FL=gb:NM_002639.1 gb:U04313.1	204855_at
spondin 1, (f-spondin) extracellular matrix protein SEQ ID NO: 54 (DNA)	Consensus includes gb:AI885290 /FEA=EST /DB_XREF=gi:5590454 /DB_XREF=est:wl92a04.x1 /CLONE=IMAGE:2432334 /UG=Hs.5378 spondin 1, (f-spondin) extracellular matrix protein	213994_s_at
superoxide dismutase 3, extracellular SEQ ID NOS: 55 (DNA) and 167 (amino acid)	gb:NM_003102.1 /DEF=Homo sapiens superoxide dismutase 3, extracellular (SOD3), mRNA. /FEA=mRNA /GEN=SOD3 /PROD=superoxide dismutase 3, extracellular /DB_XREF=gi:4507150 /UG=Hs.2420 superoxide dismutase 3, extracellular /FL=gb:J02947.1 gb:NM_003102.1	205236_x_at
tumor necrosis factor receptor superfamily, member 14 (herpesvirus entry mediator)	gb:BC002794.1 /DEF=Homo sapiens, tumor necrosis factor receptor superfamily, member 14 (herpesvirus entry mediator), clone MGC:3753, mRNA, complete cds. /FEA=mRNA /PROD=tumor necrosis factor receptor superfamily,member 14 (herpesvirus	209354_at

SEQ ID NOS: 56 (DNA) and 168 (amino acid)	entry mediator) /DB_XREF=gi:12803894 /UG=Hs.279899 tumor necrosis factor receptor superfamily, member 14 (herpesvirus entry mediator) /FL=gb:BC002794.1 gb:U70321.1 gb:U81232.1 gb:NM_003820.1 gb:AF153978.1	
tumor necrosis factor receptor superfamily, member 6 SEQ ID NOS: 57 (DNA) and 169 (amino acid)	gb:NM_000043.1 /DEF=Homo sapiens tumor necrosis factor receptor superfamily, member 6 (TNFRSF6), mRNA. /FEA=mRNA /GEN=TNFRSF6 /PROD=apoptosis (APO-1) antigen 1 /DB_XREF=gi:4507582 /UG=Hs.82359 tumor necrosis factor receptor superfamily, member 6 /FL=gb:M67454.1 gb:NM_000043.1	204781_s_at
zinc finger protein 137 (clone pHZ-30) SEQ ID NOS: 58 (DNA) and 170 (amino acid)	gb:NM_003438.1 /DEF=Homo sapiens zinc finger protein 137 (clone pHZ-30) (ZNF137), mRNA. /FEA=mRNA /GEN=ZNF137 /PROD=zinc finger protein 137 (clone pHZ-30) /DB_XREF=gi:4507988 /UG=Hs.151689 zinc finger protein 137 (clone pHZ-30) /FL=gb:NM_003438.1 gb:U09414.1	207394_at
hypothetical protein FLJ22233 SEQ ID NO: 59 (DNA)	Consensus includes gb:AI339568 /FEA=EST /DB_XREF=gi:4076495 /DB_XREF=est:qk67e10.x1 /CLONE=IMAGE:1874058 /UG=Hs.286194 hypothetical protein FLJ22233 /FL=gb:NM_024959.1	222727_s_at
regenerating gene type IV SEQ ID NOS: 60 (DNA) and 171 (amino acid)	gb:AY007243.1 /DEF=Homo sapiens regenerating gene type IV mRNA, complete cds. /FEA=mRNA /PROD=regenerating gene type IV /DB_XREF=gi:12621025 /UG=Hs.105484 Homo sapiens regenerating gene type IV mRNA, complete cds /FL=gb:AY007243.1	223447_at
Homo sapiens cDNA: FLJ21962 fis, clone HEP05564 SEQ ID NO: 61 (DNA)	Consensus includes gb:AK025615.1 /DEF=Homo sapiens cDNA: FLJ21962 fis, clone HEP05564. /FEA=mRNA /DB_XREF=gi:10438186 /UG=Hs.7567 Homo sapiens cDNA: FLJ21962 fis, clone HEP05564	225285_at
ESTs SEQ ID NO: 62 (DNA)	Consensus includes gb:N37023 /FEA=EST /DB_XREF=gi:1158165 /DB_XREF=est:yy40d03.s1 /CLONE=IMAGE:273701 /UG=Hs.235883 ESTs	225407_at
phosphoprotein associated with glycosphingolipid-	Consensus includes gb:AK000680.1 /DEF=Homo sapiens cDNA FLJ20673 fis, clone KAIA4464. /FEA=mRNA	225626_at

enriched microdomains SEQ ID NOS: 63 (DNA) and 172 (amino acid)	/DB_XREF=gi:7020924 /UG=Hs.266175 phosphoprotein associated with GEMs /FL=gb:AF240634.1 gb:NM_018440.1	
prostate cancer associated protein 7 SEQ ID NO: 64 (DNA)	Consensus includes gb:AA633076 /FEA=EST /DB_XREF=gi:2556490 /DB_XREF=est:nq38a06.s1 /CLONE=IMAGE:1146130 /UG=Hs.27495 prostate cancer associated protein 7	226167_at
Homo sapiens, Similar to RIKEN cDNA 1110060O18 gene, clone MGC:17236 IMAGE:3864137, mRNA, complete cds SEQ ID NO: 65 (DNA)	Consensus includes gb:AA524690 /FEA=EST /DB_XREF=gi:2265618 /DB_XREF=est:ng38e07.s1 /CLONE=IMAGE:937092 /UG=Hs.294143 ESTs, Weakly similar to predicted using Genefinder C.elegans	226168_at
hypothetical protein FLJ20209 SEQ ID NO: 66 (DNA)	Consensus includes gb:BF111925 /FEA=EST /DB_XREF=gi:10941704 /DB_XREF=est:7l38g05.x1 /CLONE=IMAGE:3523784 /UG=Hs.3685 hypothetical protein FLJ20209	226171_at
Homo sapiens mRNA for KIAA1190 protein, partial cds SEQ ID NOS: 67 (DNA) and 173 (amino acid)	Consensus includes gb:AA532640 /FEA=EST /DB_XREF=gi:2276894 /DB_XREF=est:nj17c04.s1 /CLONE=IMAGE:986598 /UG=Hs.206259 Homo sapiens mRNA for KIAA1190 protein, partial cds	226484_at
KIAA1543 protein SEQ ID NOS: 68 (DNA) and 174 (amino acid)	Consensus includes gb:AB040976.1 /DEF=Homo sapiens mRNA for KIAA1543 protein, partial cds. /FEA=mRNA /GEN=KIAA1543 /PROD=KIAA1543 protein /DB_XREF=gi:7959352 /UG=Hs.17686 KIAA1543 protein	226494_at
hypothetical protein MGC20702 SEQ ID NO: 69 (DNA)	Consensus includes gb:AK002203.1 /DEF=Homo sapiens cDNA FLJ11341 fis, clone PLACE1010786. /FEA=mRNA /DB_XREF=gi:7023932 /UG=Hs.10260 Homo sapiens cDNA FLJ11341 fis, clone PLACE1010786	226992_at
Homo sapiens cDNA FLJ13137 fis, clone NT2RP3003150	Consensus includes gb:AA129774 /FEA=EST /DB_XREF=gi:1690185 /DB_XREF=est:zl16h09.s1	227019_at

SEQ ID NO: 70 (DNA)	/CLONE=IMAGE:502145 /UG=Hs.288905 Homo sapiens cDNA FLJ13137 fis, clone NT2RP3003150	
hypothetical protein FLJ23563 SEQ ID NO: 71 (DNA)	Consensus includes gb:AW138767 /FEA=EST /DB_XREF=gi:6143085 /DB_XREF=est:UI-H-BI1-aep-a-12-0-UI.s1 /CLONE=IMAGE:2719799 /UG=Hs.274256 hypothetical protein FLJ23563	227180_at
ESTs SEQ ID NO: 72 (DNA)	Consensus includes gb:AW264333 /FEA=EST /DB_XREF=gi:6641075 /DB_XREF=est:xq98e01.x1 /CLONE=IMAGE:2758680 /UG=Hs.21835 ESTs	227320_at
ESTs SEQ ID NO: 73 (DNA)	Consensus includes gb:BF589359 /FEA=EST /DB_XREF=gi:11681683 /DB_XREF=est:nab25d01.x1 /CLONE=IMAGE:3266737 /UG=Hs.13256 ESTs	227354_at
Homo sapiens, Similar to RIKEN cDNA 1810037C20 gene, clone MGC:21481 IMAGE:3852062, mRNA, complete cds SEQ ID NO: 74 (DNA)	Consensus includes gb:AW001287 /FEA=EST /DB_XREF=gi:5848203 /DB_XREF=est:wu27e06.x1 /CLONE=IMAGE:2521282 /UG=Hs.61265 ESTs, Weakly similar to G786_HUMAN PROTEIN GS3786 H.sapiens	227676_at
ESTs, Weakly similar to JX0331 laurate omega-hydroxylase [H.sapiens] SEQ ID NO: 75 (DNA)	Consensus includes gb:AA557324 /FEA=EST /DB_XREF=gi:2327801 /DB_XREF=est:nl81a02.s1 /CLONE=IMAGE:1057034 /UG=Hs.26040 ESTs, Weakly similar to fatty acid omega-hydroxylase H.sapiens	227702_at
Homo sapiens cDNA: FLJ22063 fis, clone HEP10326 SEQ ID NO: 76 (DNA)	Consensus includes gb:T86159 /FEA=EST /DB_XREF=gi:714511 /DB_XREF=est:yd84h07.s1 /CLONE=IMAGE:114973 /UG=Hs.10450 Homo sapiens cDNA: FLJ22063 fis, clone HEP10326	227724_at
GalNAc alpha-2, 6-sialyltransferase I, long form SEQ ID NOS: 77 (DNA) and 175 (amino acid)	Consensus includes gb:Y11339.2 /DEF=Homo sapiens mRNA for GalNAc alpha-2, 6-sialyltransferase I, long form. /FEA=mRNA /PROD=GalNAc alpha-2,6-sialyltransferase I /DB_XREF=gi:7576275 /UG=Hs.105352 GalNAc alpha-2, 6-sialyltransferase I, long form	227725_at

ESTs, Weakly similar to JE0350 Anterior gradient-2 [H.sapiens] SEQ ID NO: 78 (DNA)	Consensus includes gb:AI827789 /FEA=EST /DB_XREF=gi:5448449 /DB_XREF=est:wf33a07.x1 /CLONE=IMAGE:2357364 /UG=Hs.100686 ESTs, Weakly similar to JE0350 Anterior gradient-2 H.sapiens	228241_at
ESTs SEQ ID NO: 79 (DNA)	Consensus includes gb:AI700341 /FEA=EST /DB_XREF=gi:4988241 /DB_XREF=est:wd06e10.x1 /CLONE=IMAGE:2327370 /UG=Hs.110406 ESTs	228653_at
ESTs SEQ ID NO: 80 (DNA)	Consensus includes gb:BG494007 /FEA=EST /DB_XREF=gi:13455521 /DB_XREF=est:602542289F1 /CLONE=IMAGE:4673182 /UG=Hs.203213 ESTs	228716_at
anterior gradient 2 (Xenepus laevis) homolog SEQ ID NO: 81 (DNA)	Consensus includes gb:AI922323 /FEA=EST /DB_XREF=gi:5658287 /DB_XREF=est:wn90h03.x1 /CLONE=IMAGE:2453141 /UG=Hs.293380 ESTs	228969_at
Homo sapiens cDNA: FLJ23331 fis, clone HEP12664 SEQ ID NO: 82 (DNA)	Consensus includes gb:AK026984.1 /DEF=Homo sapiens cDNA: FLJ23331 fis, clone HEP12664. /FEA=mRNA /DB_XREF=gi:10439980 /UG=Hs.50742 Homo sapiens cDNA: FLJ23331 fis, clone HEP12664	229021_at
ESTs SEQ ID NO: 83 (DNA)	Consensus includes gb:AI559300 /FEA=EST /DB_XREF=gi:4509505 /DB_XREF=est:tq43d03.x1 /CLONE=IMAGE:2211557 /UG=Hs.294140 ESTs	229331_at
hypothetical protein SEQ ID NO: 84 (DNA)	Consensus includes gb:AI830823 /FEA=EST /DB_XREF=gi:5451416 /DB_XREF=est:wj52b06.x1 /CLONE=IMAGE:2406419 /UG=Hs.95549 hypothetical protein	229439_s_at
ESTs SEQ ID NO: 85 (DNA)	Consensus includes gb:BF431989 /FEA=EST /DB_XREF=gi:11444103 /DB_XREF=est:nab84a05.x1 /CLONE=IMAGE:3274280 /UG=Hs.203213 ESTs	229657_at
ESTs SEQ ID NO: 86 (DNA)	Consensus includes gb:BF589413 /FEA=EST /DB_XREF=gi:11681737 /DB_XREF=est:nab26b11.x1 /CLONE=IMAGE:3267020 /UG=Hs.55501	229893_at

	ESTs	
brain-specific protein p25 alpha SEQ ID NO: 87 (DNA)	Consensus includes gb:BG055052 /FEA=EST /DB_XREF=gi:12512386 /DB_XREF=est:nac94g06.x1 /CLONE=IMAGE:3441995 /UG=Hs.29353 brain-specific protein p25 alpha	230104_s_at
ESTs, Weakly similar to MMHUE4 erythrocyte membrane protein 4.1, parent splice form [H.sapiens] SEQ ID NO: 88 (DNA)	Consensus includes gb:BF110588 /FEA=EST /DB_XREF=gi:10940278 /DB_XREF=est:7n39e12.x1 /CLONE=IMAGE:3567071 /UG=Hs.150478 ESTs, Weakly similar to KIAA0987 protein H.sapiens	230645_at
ESTs SEQ ID NO: 89 (DNA)	Consensus includes gb:BF592062 /FEA=EST /DB_XREF=gi:11684386 /DB_XREF=est:7n98h06.x1 /CLONE=IMAGE:3572962 /UG=Hs.233890 ESTs	230760_at
hepatocyte nuclear factor 4, alpha SEQ ID NO: 90 (DNA)	Consensus includes gb:AI032108 /FEA=EST /DB_XREF=gi:3250320 /DB_XREF=est:ow92d11.x1 /CLONE=IMAGE:1654293 /UG=Hs.54424 hepatocyte nuclear factor 4, alpha	230914_at
ESTs SEQ ID NO: 91 (DNA)	Consensus includes gb:AW203959 /FEA=EST /DB_XREF=gi:6503431 /DB_XREF=est:UI-H-BI1-aeu-b-12-0-UI.s1 /CLONE=IMAGE:2720590 /UG=Hs.149532 ESTs	230944_at
ESTs SEQ ID NO: 92 (DNA)	Consensus includes gb:AI139990 /FEA=EST /DB_XREF=gi:3647447 /DB_XREF=est:qa47d03.x1 /CLONE=IMAGE:1689893 /UG=Hs.134586 ESTs	231022_at
ESTs SEQ ID NO: 93 (DNA)	Consensus includes gb:AI806131 /FEA=EST /DB_XREF=gi:5392697 /DB_XREF=est:wf06c06.x1 /CLONE=IMAGE:2349802 /UG=Hs.99376 ESTs	231148_at
hypothetical protein FLJ23045 SEQ ID NO: 94 (DNA)	Consensus includes gb:AB046810.1 /DEF=Homo sapiens mRNA for KIAA1590 protein, partial cds. /FEA=mRNA /GEN=KIAA1590 /PROD=KIAA1590 protein /DB_XREF=gi:10047254 /UG=Hs.101774 hypothetical protein FLJ23045	232083_at
Homo sapiens cDNA:	Consensus includes gb:AK026404.1	232321_at

FLJ22751 fis, clone KAIA0483, highly similar to AF016692 Homo sapiens small intestinal mucin (MUC3) mRNA SEQ ID NO: 95 (DNA)	/DEF=Homo sapiens cDNA: FLJ22751 fis, clone KAIA0483, highly similar to AF016692 Homo sapiens small intestinal mucin (MUC3) mRNA. /FEA=mRNA /DB_XREF=gi:10439257 /UG=Hs.271819 Homo sapiens cDNA: FLJ22751 fis, clone KAIA0483, highly similar to AF016692 Homo sapiens small intestinal mucin (MUC3) mRNA	
Homo sapiens PAC clone RP5-855D21 SEQ ID NOS: 96 (DNA), 176 (amino acid), 177 (amino acid), and 178 (amino acid)	Consensus includes gb:AC004908 /DEF=Homo sapiens PAC clone RP5-855D21 /FEA=CDS_3 /DB_XREF=gi:4156179 /UG=Hs.249181 Homo sapiens PAC clone RP5-855D21	232641_at
putative microtubule-binding protein SEQ ID NO: 97 (DNA)	Consensus includes gb:AJ251708.1 /DEF=Homo sapiens partial mRNA for putative microtubule-binding protein. /FEA=mRNA /PROD=putative microtubule-binding protein /DB_XREF=gi:6491740 /UG=Hs.326544 putative microtubule-binding protein	234669_x_at
ESTs SEQ ID NO: 98 (DNA)	Consensus includes gb:AI741469 /FEA=EST /DB_XREF=gi:5109757 /DB_XREF=est:wg11b01.x1 /CLONE=IMAGE:2364745 /UG=Hs.57787 ESTs	234970_at
ESTs SEQ ID NO: 99 (DNA)	Consensus includes gb:AI417897 /FEA=EST /DB_XREF=gi:4261401 /DB_XREF=est:tg55b06.x1 /CLONE=IMAGE:2112659 /UG=Hs.235860 ESTs	235444_at
ESTs SEQ ID NO: 100 (DNA)	Consensus includes gb:AA827649 /FEA=EST /DB_XREF=gi:2900090 /DB_XREF=est:od01a12.s1 /CLONE=IMAGE:1357918 /UG=Hs.105317 ESTs	235515_at
ESTs SEQ ID NO: 101 (DNA)	Consensus includes gb:AI493909 /FEA=EST /DB_XREF=gi:4394912 /DB_XREF=est:qz94e02.x1 /CLONE=IMAGE:2042234 /UG=Hs.6131 ESTs	235562_at
ESTs SEQ ID NO: 102 (DNA)	Consensus includes gb:AV741130 /FEA=EST /DB_XREF=gi:10858711 /DB_XREF=est:AV741130 /CLONE=CBCATB06 /UG=Hs.173704	235651_at

	ESTs, Moderately similar to ALU8_HUMAN ALU SUBFAMILY SX SEQUENCE CONTAMINATION WARNING ENTRY H.sapiens	
ESTs, Weakly similar to I38588 reverse transcriptase homolog [H.sapiens] SEQ ID NO: 103 (DNA)	Consensus includes gb:AI864053 /FEA=EST /DB_XREF=gi:5528160 /DB_XREF=est:wj55h10.x1 /CLONE=IMAGE:2406787 /UG=Hs.39972 ESTs, Weakly similar to I38588 reverse transcriptase homolog H.sapiens	235678_at
ESTs SEQ ID NO: 104 (DNA)	Consensus includes gb:AW339510 /FEA=EST /DB_XREF=gi:6836136 /DB_XREF=est:xz91h08.x1 /CLONE=IMAGE:2871615 /UG=Hs.42722 ESTs	235866_at
ESTs SEQ ID NO: 105 (DNA)	Consensus includes gb:AI076192 /FEA=EST /DB_XREF=gi:3405370 /DB_XREF=est:oz01g07.x1 /CLONE=IMAGE:1674108 /UG=Hs.131933 ESTs	236422_at
ESTs SEQ ID NO: 106 (DNA)	Consensus includes gb:AL044570 /FEA=EST /DB_XREF=gi:5432785 /DB_XREF=est:DKFZp434L082_s1 /CLONE=DKFZp434L082 /UG=Hs.147975 ESTs	236548_at
ESTs SEQ ID NO: 107 (DNA)	Consensus includes gb:AI968097 /FEA=EST /DB_XREF=gi:5764915 /DB_XREF=est:wu13a12.x1 /CLONE=IMAGE:2516830 /UG=Hs.131360 ESTs	237835_at
ESTs SEQ ID NO: 108 (DNA)	Consensus includes gb:AI733801 /FEA=EST /DB_XREF=gi:5054914 /DB_XREF=est:qk39c04.x5 /CLONE=IMAGE:1871334 /UG=Hs.146186 ESTs	237923_at
ESTs SEQ ID NO: 109 (DNA)	Consensus includes gb:BF594323 /FEA=EST /DB_XREF=gi:11686647 /DB_XREF=est:7h79g07.x1 /CLONE=IMAGE:3322236 /UG=Hs.158989 ESTs	238103_at
Homo sapiens, clone MGC:16402 IMAGE:3940360, mRNA, complete cds SEQ ID NO: 110 (DNA)	Consensus includes gb:T69015 /FEA=EST /DB_XREF=gi:680163 /DB_XREF=est:yc31f04.s1 /CLONE=IMAGE:82303 /UG=Hs.192728 ESTs	238422_at

ESTs SEQ ID NO: 111 (DNA)	Consensus includes gb:AA502384 /FEA=EST /DB_XREF=gi:2237351 /DB_XREF=est:ne27f11.s1 /CLONE=IMAGE:898605 /UG=Hs.151529 ESTs	238956_at
ESTs SEQ ID NO: 112 (DNA)	Consensus includes gb:AI739241 /FEA=EST /DB_XREF=gi:5101222 /DB_XREF=est:wi14h02.x1 /CLONE=IMAGE:2390259 /UG=Hs.171480 ESTs	238984_at
ESTs SEQ ID NO: 113 (DNA)	Consensus includes gb:AA088446 /FEA=EST /DB_XREF=gi:1633958 /DB_XREF=est:zl89f04.s1 /CLONE=IMAGE:511807 /UG=Hs.170298 ESTs	239065_at
ESTs SEQ ID NO: 114 (DNA)	Consensus includes gb:AI493046 /FEA=EST /DB_XREF=gi:4394049 /DB_XREF=est:qz49b04.x1 /CLONE=IMAGE:2030191 /UG=Hs.146133 ESTs	239148_at
ESTs SEQ ID NO: 115 (DNA)	Consensus includes gb:AI243098 /FEA=EST /DB_XREF=gi:3838495 /DB_XREF=est:qh26e03.x1 /CLONE=IMAGE:1845820 /UG=Hs.178398 ESTs	239966_at
ESTs, Weakly similar to A49175 Motch B protein - mouse [M.musculus] SEQ ID NO: 116 (DNA)	Consensus includes gb:AI633523 /FEA=EST /DB_XREF=gi:4684853 /DB_XREF=est:th68b11.x1 /CLONE=IMAGE:2123805 /UG=Hs.44705 ESTs	240106_at
ESTs SEQ ID NO: 117 (DNA)	Consensus includes gb:AI300126 /FEA=EST /DB_XREF=gi:3959472 /DB_XREF=est:qn54f02.x1 /CLONE=IMAGE:1902075 /UG=Hs.257858 ESTs	240830_at
ESTs SEQ ID NO: 118 (DNA)	Consensus includes gb:AI917390 /FEA=EST /DB_XREF=gi:5637245 /DB_XREF=est:ts79a05.x1 /CLONE=IMAGE:2237456 /UG=Hs.99415 ESTs	240964_at
betacellulin SEQ ID NO: 119 (DNA)	Consensus includes gb:AI620677 /FEA=EST /DB_XREF=gi:4629803 /DB_XREF=est:tu85e09.x1 /CLONE=IMAGE:2257864 /UG=Hs.154191 ESTs	241412_at
ESTs	Consensus includes gb:H05025 /FEA=EST	241874_at

SEQ ID NO: 120 (DNA)	/DB_XREF=gi:868577 /DB_XREF=est:yl74g12.s1 /CLONE=IMAGE:43864 /UG=Hs.323767 ESTs	
ESTs SEQ ID NO: 121 (DNA)	Consensus includes gb:AW024656 /FEA=EST /DB_XREF=gi:5878186 /DB_XREF=est:wu78h05.x1 /CLONE=IMAGE:2526201 /UG=Hs.233382 ESTs, Moderately similar to AF119917 62 PRO2822 H.sapiens	242358_at
ESTs SEQ ID NO: 122 (DNA)	Consensus includes gb:BF696216 /FEA=EST /DB_XREF=gi:11981624 /DB_XREF=est:602124536F1 /CLONE=IMAGE:4281632 /UG=Hs.188724 ESTs	242626_at
ESTs SEQ ID NO: 123 (DNA)	Consensus includes gb:N57929 /FEA=EST /DB_XREF=gi:1201819 /DB_XREF=est:yv61e06.s1 /CLONE=IMAGE:247234 /UG=Hs.48100 ESTs	242978_x_at
ESTs, Weakly similar to ALU1_HUMAN ALU SUBFAMILY J SEQUENCE CONTAMINATION WARNING ENTRY [H.sapiens] SEQ ID NO: 124 (DNA)	Consensus includes gb:AI457984 /FEA=EST /DB_XREF=gi:4312002 /DB_XREF=est:tj66a04.x1 /CLONE=IMAGE:2146446 /UG=Hs.165900 ESTs, Weakly similar to ALUC_HUMAN !!!! ALU CLASS C WARNING ENTRY !!! H.sapiens	243729_at
ESTs SEQ ID NO: 125 (DNA)	Consensus includes gb:AA581439 /FEA=EST /DB_XREF=gi:2359211 /DB_XREF=est:nh13c10.s1 /CLONE=IMAGE:952242 /UG=Hs.152328 ESTs	244650_at

Biological Validation of Biomarker Candidates: Modulation of Expression by Treatment with Ligands for EGFR or by Treatment with Inhibitors for EGFR

To validate the significance of the biomarker candidates to predict the activity of the EGFR pathway and thereby the sensitivity of cancer cell to inhibition of EGFR by therapy, genes that would be regulated by the EGFR pathway were identified. Demonstration of that property for the EGFR biomarker candidates described above would add additional credibility as it would link these genes functionally to the EGFR pathway. Colon cancer and a lung cancer cell lines were treated with epidermal

growth factor, in the absence of serum or, in the presence of serum with the EGFR modulator BMS-461453 or the EGFR modulator cetuximab (also known as C225, a chimeric monoclonal EGFR antibody). To identify genes induced by epidermal growth factor, serum starved cells were treated with 20ng/ml EGF for 0.5, 6, and 18 hours. Control cells were treated with media alone. The expression profiling was performed, and data was analyzed using GeneChip® Expression Analysis software MAS 5.0 (Affymetrix, Santa Clara, California).

Genes inhibited by EGFR antagonists were identified by treating cells in the presence of 10% serum with 0.5uM of BMS-461453 or 1ug/ml or 5ug/ml of C225 for 6 and 24 hours. Cells exposed to 0.05% DMSO were used as the experimental control. Expression profiling was performed, and data were analyzed using GeneChip® Expression Analysis software MAS 5.0.

The gene expression of the inhibitor or EGFR treated cell lines was compared pair-wise to the untreated controls. Polynucleotides from the biomarker list, in which expression was increased two fold with EGFR exposure or decreased two fold with EGFR inhibitor treatment compared to the untreated controls, were considered to be modulated by EGFR. These biomarkers are provided in Table 4. Examples of the biomarkers include EphA1, B-cell translocation gene 2, prostaglandin-endoperoxide synthase 2 and serine (or cysteine) proteinase inhibitor (clade B), which are highly expressed in sensitive cells and up regulated by treatment with EGFR. On the other hand, spondin 1, talin 2 and nuclear receptor subfamily 3 are genes whose expression levels correlate with sensitivity or resistance of colon cancer cell lines and are consistently down regulated by treatment with EGFR inhibitors BMS-461453 and C225. It appears that these biomarkers are likely to be directly or indirectly involved in the EGFR signaling pathway, based on their expression modulation by EGF or EGFR inhibitor treatment.

Identification of Top Biomarkers

In an attempt to further prioritize biomarkers for use in predicting response of cancer cells to treatment with one or more EGFR modulators, the following filter criteria were used on the Table 4 biomarkers to identify a total of fourteen biomarkers (Table 5) as the top biomarkers:

(1) results from the highly significant correlation of gene expression with IC_{50}: A p-value < 0.01 in the student TTEST or a Pearson value < – 0.6 described above;

(2) results from the modulation of expression by EGFR ligand and/or EGFR inhibitor treatment described above; and

(3) biomarkers supported by literature revealing a direct relationship between the EGFR pathway and the biomarkers.

TABLE 5 - Top Fourteen Biomarkers

Biomarker Name	Literature Support Citation	Induced by EGF/ Inhibited by EGFR antagonist
mucin 2, intestinal/tracheal (MUC2)	J Biol Chem. 2002 Aug 30;277(35):32258-67	Expression inhibited 2 fold by EGFR antagonist in GEO colon cancer cell line
intestinal mucin 3 (MUC3)	No	Expression inhibited 2 fold by EGFR antagonist in GEO colon cancer cell line
Homo sapiens cystic fibrosis transmembrane conductance regulator ATP-binding cassette (sub-family C, member 7) (CFTR)	No	Expression stimulated 2 fold by EGFR in H292 lung cancer cell line
f-spondin (KIAA0762) protein	No	Expression inhibited 2 fold by EGFR antagonist in LOVO colon cancer cell line
3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2	J Invest Dermatol. 2000 Jan;114(1):83-7	Expression stimulated 3 fold by EGFR in H292 lung cancer cell line
serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 5 (SERPINB5	Electrophoresis. 2001 Aug;22(14):3001-8.	Expression stimulated 2 fold by EGFR in H292 lung cancer cell line
BTG family, member 2 (BTG2)	No	Expression stimulated 2 fold by EGFR in H292 lung cancer cell line
talin 2 (TLN2)	No	Expression inhibited 2 fold by EGFR antagonist in GEO colon cancer cell line
arachidonic acid	J Biol Chem. 1994 Aug	no

epoxygenase	26;269(34):21786-92.	
prostaglandin G/H synthase and cyclooxygenase	J Biol Chem. 1994 Aug 26;269(34):21786-92.	Expression stimulated 6 fold by EGFR in H292 lung cancer cell line
EphA1 (EPHA1)	No	Expression stimulated 2 fold by EGFR in CACO2 colon cancer cell line
hemoglobin, alpha 1 (HBA1)	No	Expression inhibited 2 fold by EGFR antagonist in GEO colon cancer cell line
bone morphogenetic protein 2	Development 2000 Nov;127(22):4993-5005	no
betacellulin (BTC)*	Biochem Biophys Res Commun. 2002 Jun 28;294(5):1040-6	no

*The gene betacellulin showed counter regulation with EGFR expression as defined for the EGFR-A list but had just a p value of 0.04 in the Student's TTest for correlation with IC_{50}. It was still selected as a top biomarker for the strong literature support, as betacellulin is one of the published ligands of EGFR.

Utility of Biomarkers

Polynucleotides that correlate to a specific property of a biological system can be used to make predictions about that biological system and other biological systems. To show the predictive utility of biomarkers that correlate to EGFR modulator sensitivity and resistance, these polynucleotides were tested for their ability to predict the response of twenty two colon cancer cell lines to a small molecule EGFR modulator.

The invention includes single biomarkers including, for example, the fourteen top biomarkers which were tested in a voting scheme. For that purpose, the mean expression value was calculated for all fourteen biomarkers. Colon cancer cell lines which showed an expression level above the mean were then voted to be sensitive, and colon cancer cell lines with expression levels below the mean were voted to be resistant. After this procedure, the voting was compared to the actual sensitivity/resistance status according to the definition based on IC_{50} (see above) and an error rate was calculated. The error rates of the fourteen top biomarkers are shown in Table 6.

TABLE 6 - Error Rates of Fourteen Top Biomarkers

Biomarker Name	Pearsons value	TTEST P value	Prediction error rate
mucin 2,	-0.531	0.0083	20%

intestinal/tracheal (MUC2)			
intestinal mucin 3 (MUC3)	-0.639	0.0004	11.72%
Homo sapiens cystic fibrosis transmembrane conductance regulator ATP-binding cassette (sub-family C, member 7) (CFTR)	-0.646	9E-05	5.9%
f-spondin (KIAA0762) protein	-0.622	0.0004	12.8%
3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2	-0.575	0.0029	21.75%
serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 5 (SERPINB5	-0.62	0.0028	21.75%
BTG family, member 2 (BTG2)	-0.544	0.0042	20.5%
talin 2 (TLN2)	-0.874	3E-05	8.8%
EphA1 (EPHA1)	-0.647	0.0021	22%
hemoglobin, alpha 1 (HBA1)	-0.744	8E-05	20%
bone morphogenetic protein 2	-0.555	0.0091	31.8%
betacellulin (BTC)	-0.536	0.047	43.5%

The biomarkers talin, the Cystic fibrosis conductance regulator (CFTR), and mucin 3 were the best single biomarkers with error rates below 12%.

5

EXAMPLES:

EXAMPLE 1 - METHODS

IC_{50} determination--*in vitro* cytotoxicity assay

A small molecule EGFR inhibitor, erlotinib HCl (BMS-461453), was tested for cytoxicity *in vitro* against a panel of twenty-two human colon cancer cell lines

available from the American Type Culture Collection. Cytotoxicity was assessed in cells by MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphenyl)-2H-tetrazolium, inner salt) assay (T.L. Riss et al., 1992, *Mol. Biol. Cell*, 3 (Suppl.):184a).

To carry out the assays, the colon cells were plated at 4,000 cell/well in 96 well microtiter plates and 24 hours later serial diluted drugs were added. The concentration range for the EGFR inhibitor was from 5 μg/ml to 0.0016 μg/ml (roughly 10 μM to 0.0032 μM). The cells were incubated at 37 °C for 72 hours at which time the tetrazolium dye MTS (333 μg/ml final concentration) in combination with the electron coupling agent phenazine methosulfate (25 μM final concentration) was added. A dehydrogenase enzyme in live cells reduces the MTS to a form that absorbs light at 492 nm that can be quantified spectrophotometrically. The greater the absorbency, the greater the number of live cells. The results were expressed as an IC_{50}, which is the drug concentration required to inhibit cell proliferation (i.e., absorbance at 450 nm) to 50% of that of untreated control cells. The mean IC_{50} and standard deviation (SD) from multiple tests for each cell line were calculated.

Resistant/sensitive classification

The cell lines with IC_{50} below 6 μM were defined as sensitive to the EGFR inhibitor, whereas those with IC_{50} above 6 μM were considered to be resistant. The resistant/sensitive classification are shown above in Table 1, with five cell lines classified as sensitive and seventeen cell lines classified as resistant.

Gene expression profiling

The colon cells were grown using standard cell culture conditions: RPMI 1640 supplemented to contain 10% fetal bovine serum, 100 IU/ml penicillin, 100 mg/ml streptomycin, 2 mM L-glutamine and 10 mM Hepes (all from GibcoBRL, Rockville, Maryland). RNA was isolated from 50-70% confluent cells or drug-treated cells using the RNeasy™ kits commercially available from Qiagen (Valencia, California). Quality of the RNA was checked by measuring the 28s:18s ribosomal RNA ratio using Agilent 2100 bioanalyzer (Agilent, Technologies, Rockville, Maryland). Concentration of total RNA was determined spectrophotometrically. 10

μg of total RNA from each cell line was used to prepare biotinylated probe according to the Affymetrix Genechip® Expression Analysis Technical Manual, 2001. Targets were hybridized to Affymetrix high density oligonucleotide array human HG-U133 set chips (Affymetrix, Santa Clara, California). Arrays were then washed, and stained using the GeneChip Fluidics station according to the manufacture's instructions. The HG-U133 set consisting of two GeneChip® arrays contains nearly 45,000 probe sets representing more than 39,000 transcripts derived from approximately 33,000 well-substantiated human genes.

Preprocessing of microarray data for selecting biomarkers

Scanned image files were visually inspected for artifacts and analyzed with GeneChip® Expression Analysis software MAS 5.0 (Affymetrix, Santa Clara, California). The "Detection Call" (see Affymetrix manual) was used to determine whether a transcript was detected within one sample, as well as the "Signal" (see Affymetrix Genechip® Expression Analysis Technical Manual, 2001) which measured the relative abundance of a transcript. The trimmed mean intensity for each chip was scaled to 1,500 (see Affymetrix manual) in order to account for any minor differences in global chip intensity, so that the overall expression level for each cell line is comparable. Affymetrix control sequences were removed prior to analysis.

Induction Studies of colon and breast cell lines with EGFR inhibitors or EGFR ligand and selection of genes modulated by the inductions

The five colon cell lines and one lung cell line indicated with asterisks in Table 1 were used in the drug induction study. Three of the colon cell lines express EGFR and are sensitive to the EGFR inhibitor BMS-461453. The SW480 cell line, while expressing EGFR, is insensitive to the EGFR inhibitor, and the COLO320_DM does not express EGFR and is EGFR inhibitor resistant. The lung cancer cell line H292 expresses EGFR, but its sensitivity status is unknown. Cells were seeded in a 10 cm^2 culture plate with the medium described above and cultured for 24 hours.

For the EGF induction studies, the colon cell line CACO2 and the lung cancer H292 cell line were washed 2X PBS, and the media was changed to RPMI without serum. The next day the cells were treated with 20 ng/ml EGF, and eventually lysed

for RNA isolation 0.5, 6 and 18 hours post treatment. Gene expression was profiled as described below.

EGFR inhibition studies were conducted on the colon cell lines GEO, CCD33-CO, SW480 and COLO320DM. The expression profiling was performed as described above and data was analyzed using GeneChip® Expression Analysis software MAS 5.0. The expression data of EGFR inhibitor treated cell lines were compared pair-wise to that of untreated same cell line. A change was considered significant if a two fold difference in expression was demonstrated between the treated and the untreated control. Analysis was done for all four cell lines to compare the gene expression with or without EGFR inhibitor treatment.

EXAMPLE 2 - RT-PCR EXPRESSION PROFILING

RNA quantification was performed using the SYBR Green real-time PCR. The SYBR Green real-time PCR assay is one of the most precise methods for assaying the concentration of nucleic acid templates.

RNA can be prepared using standard methods, preferably, employing the RNeasy Kit commercially available from Qiagen (Valencia, California). cDNA template for real-time PCR can be generated using the Superscript™ First Strand Synthesis system for RT-PCR. SYBR Green real-time PCR reactions are prepared as follows: the reaction mix contains 20 ng first strand cDNA; 50 nM Forward Primer; 50 nM Reverse Primer; 0.75X SYBR Green I (Sigma); 1X SYBR Green PCR Buffer (50mMTris-HCl pH 8.3, 75 mM KCl); 10% DMSO; 3 mM $MgCl_2$; 300 μM each dATP, dGTP, dTTP, dCTP; 1 U Platinum® Taq DNA Polymerase High Fidelity (Cat# 11304-029; Life Technologies; Rockville, Maryland). Real-time PCR is performed using an Applied Biosystems 5700 Sequence Detection System. Conditions are 95 °C for 10 minutes (denaturation and activation of Platinum® Taq DNA Polymerase), 40 cycles of PCR (95 °C for 15 seconds, 60 °C for 1 minute). PCR products are analyzed for uniform melting using an analysis algorithm built into the 5700 Sequence Detection System.

cDNA quantification used in the normalization of template quantity is performed using SYBR Green real-time PCR. Expression of EGFR is normalized to GAPDH expression as described below.

The sequences for the GAPDH oligonucleotides used in the SYBR Green real-time PCR reactions are:

GAPDH-F: 5'-AGCCGAGCCACATCGCT-3' (SEQ ID NO: 191)

GAPDH-R: 5'-GTGACCAGGCGCCCAATAC-3' (SEQ ID NO: 192)

The sequences for the EGFR oligonucleotides used in the SYBR Green real-time PCR reactions are:

EGFR-F: 5'- GCGTCTCTTGCCGGAATGT-3' (SEQ ID NO: 193)

EGFR-R: 5'- AGCCGAGGCAGGGAATGCGTG-3' (SEQ ID NO: 194)

The Sequence Detection System generates a Ct (threshold cycle) value that is used to calculate a concentration for each input cDNA template. cDNA levels for each gene of interest are normalized to GAPDH cDNA levels to compensate for variations in total cDNA quantity in the input sample. This is done by generating GAPDH Ct values for each cell line. Ct values for the gene of interest and GAPDH are inserted into a modified version of the δδCt equation (Applied Biosystems Prism® 5700 Sequence Detection System User Manual) which is used to calculate a GAPDH normalized relative cDNA level for each specific cDNA. The δδCt equation is: relative quantity of nucleic acid template $=2^{\delta\delta Ct} = 2^{(\delta Cta-\delta Ctb)}$, where δCta = Ct target – Ct GAPDH, and δCtb = Ct reference – Ct GAPDH.

EXAMPLE 3 - PRODUCTION OF ANTIBODIES AGAINST THE BIOMARKERS

Antibodies against the biomarkers can be prepared by a variety of methods. For example, cells expressing an biomarker polypeptide can be administered to an animal to induce the production of sera containing polyclonal antibodies directed to the expressed polypeptides. In one aspect, the biomarker protein is prepared and isolated or otherwise purified to render it substantially free of natural contaminants, using techniques commonly practiced in the art. Such a preparation is then introduced into an animal in order to produce polyclonal antisera of greater specific activity for the expressed and isolated polypeptide.

In one aspect, the antibodies of the invention are monoclonal antibodies (or protein binding fragments thereof). Cells expressing the biomarker polypeptide can be cultured in any suitable tissue culture medium, however, it is preferable to culture cells in Earle's modified Eagle's medium supplemented to contain 10% fetal bovine

serum (inactivated at about 56 °C), and supplemented to contain about 10 g/l nonessential amino acids, about 1,00 U/ml penicillin, and about 100 μg/ml streptomycin.

The splenocytes of immunized (and boosted) mice can be extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line can be employed in accordance with the invention, however, it is preferable to employ the parent myeloma cell line (SP2/0), available from the ATCC. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands et al. (1981, *Gastroenterology*, 80:225-232). The hybridoma cells obtained through such a selection are then assayed to identify those cell clones that secrete antibodies capable of binding to the polypeptide immunogen, or a portion thereof.

Alternatively, additional antibodies capable of binding to the biomarker polypeptide can be produced in a two-step procedure using anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens and, therefore, it is possible to obtain an antibody that binds to a second antibody. In accordance with this method, protein specific antibodies can be used to immunize an animal, preferably a mouse. The splenocytes of such an immunized animal are then used to produce hybridoma cells, and the hybridoma cells are screened to identify clones that produce an antibody whose ability to bind to the protein-specific antibody can be blocked by the polypeptide. Such antibodies comprise anti-idiotypic antibodies to the protein-specific antibody and can be used to immunize an animal to induce the formation of further protein-specific antibodies.

EXAMPLE 4 - IMMUNOFLUORESCENCE ASSAYS

The following immunofluorescence protocol may be used, for example, to verify EGFR biomarker protein expression on cells or, for example, to check for the presence of one or more antibodies that bind EGFR biomarkers expressed on the surface of cells. Briefly, Lab-Tek II chamber slides are coated overnight at 4 °C with 10 micrograms/milliliter (μg/ml) of bovine collagen Type II in DPBS containing calcium and magnesium (DPBS++). The slides are then washed twice with cold DPBS++ and seeded with 8000 CHO-CCR5 or CHO pC4 transfected cells in a total

volume of 125 µl and incubated at 37 °C in the presence of 95% oxygen / 5% carbon dioxide.

The culture medium is gently removed by aspiration and the adherent cells are washed twice with DPBS++ at ambient temperature. The slides are blocked with DPBS++ containing 0.2% BSA (blocker) at 0-4 °C for one hour. The blocking solution is gently removed by aspiration, and 125 µl of antibody containing solution (an antibody containing solution may be, for example, a hybridoma culture supernatant which is usually used undiluted, or serum/plasma which is usually diluted, e.g., a dilution of about 1/100 dilution). The slides are incubated for 1 hour at 0-4 °C. Antibody solutions are then gently removed by aspiration and the cells are washed five times with 400 µl of ice cold blocking solution. Next, 125 µl of 1 µg/ml rhodamine labeled secondary antibody (e.g., anti-human IgG) in blocker solution is added to the cells. Again, cells are incubated for 1 hour at 0-4 °C.

The secondary antibody solution is then gently removed by aspiration and the cells are washed three times with 400 µl of ice cold blocking solution, and five times with cold DPBS++. The cells are then fixed with 125 µl of 3.7% formaldehyde in DPBS++ for 15 minutes at ambient temperature. Thereafter, the cells are washed five times with 400 µl of DPBS++ at ambient temperature. Finally, the cells are mounted in 50% aqueous glycerol and viewed in a fluorescence microscope using rhodamine filters.